CHARACTERISATION OF SOUTH AFRICAN WHEAT GENOTYPES TO IMPROVE NUTRITIONAL QUALITY AND YIELD

by

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Exact wording of the title of the dissertation as appearing on the copies submitted for examination:

Characterisation of South African wheat genotypes to improve nutritional quality and yield

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ABSTRACT

Bread wheat (*Triticum aestivum* L.) is an important cereal crop that provides over 20% of the global calorie intake. With the world population constantly growing, yield production must increase to meet food demands. Wheat plays a significant role on nutritional and food security especially in rural areas, however, bread wheat grains are known to be inherently deficient in micronutrients, particularly Fe and Zn, which makes them important biofortification targets. To date, South African wheat genotypes have not been explored for their nutritional micronutrient variation; hence there is a need to investigate the variation of nutritional quality and its association with yield components. Bread wheat cultivars, Tugela-DN and Elands were used in this study based on their known high yield potential, resistance to insect pests and diseases as well as their good-to-excellent bread-making quality. The goal of this study was to use a doubled haploid (DH) mapping population, developed from a cross between cultivars Tugela-DN and Elands, to identify single nucleotide polymorphism (SNP) and genotyping-by-sequencing (GBS)-based markers linked to high nutritional quality and yield-related traits. This was achieved by (i) determining grain micronutrient (Fe and Zn) concentration variation in 139 lines of a DH mapping population; (ii) evaluating the mapping population for yield-related traits; (iii) determining the correlation between micronutrient and yield-related traits among the genotypes; (iv) identifying SNP GBS-based markers linked to the high minerals and yield-related traits. The analysis of variance (ANOVA) showed significant (*P<0.001)* differences between genotypes for all traits evaluated. A wide variation was observed for both GFeC and GZnC. The statistical analysis revealed significant variation for Zn concentration ($P < 0.001$) among genotypes and not significant Fe concentration. DArT-Seq was used to genotype Tugela-DN and Elands cultivars and 139 DH genotypes. Quantitative trait loci (QTL) were detected using SNP GBS-based markers on chromosome 2D, 5B, 5D, 6A, and 6B for GZnC, and on chromosome 2D, 5B, 5D and 7D for GFeC. Most QTLs identified for GFeC and GZnC shared the genomic interval and some of them also co-located with few yield-related traits. The results of this study will contribute to breeding programmes to improve nutritional quality of bread wheat and food security of the country.

Keywords: Bread wheat; Genetic variation; Linkage mapping; Nutritional quality; Quantitative trait loci; Yield-related traits

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ARC – SG	Agricultural Research Council – Small Grains
bp	Base pair(s)
CAPS	Cleaved Amplified Polymorphic Sequences
CRISPR/Cas	Clustered Regularly Interspaced Short Palindromic Repeats/Cas9
cM	Centimorgan(s)
DArT	Diversity Array Techology
DH	Doubled haploid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene-diaminetetraacetate
EST	Expressed Sequence Tags
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statistics
Fe	Iron
GBS	Genotyping-by-Sequencing
GL	Grain Length
GW	Grain Width
IWGSC	International Wheat Genome Sequencing Consortium
KNPS	Kernel Number per Spike
LOD	Logarithm of Odds

LSD	Least Significant Difference
MAS	Marker-assisted Selection
MARS	Marker-assisted Recurrent Selection
ml	Mililitre(s)
mm	Millimetre(s)
ng	Nano gram(s)
NGS	Next-Generation Sequencing
nm	Nanometre(s)
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
pH	Power of Hydrogen
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SA	South Africa
SL	Spike Length
SPS	Spikelet per Spike
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat(s)
TBE	Tris-borate-EDTA

TE	Tris-Cl/EDTA
TKW	1000-Kernel Weight
UNISA	University of South Africa
USDA	United States Department of Agriculture
v/v	Volume per volume
w/v	Weight per volume
YSL	Yellow stripe-like
Zn	Zinc
°C	Degree Celsius
µg	Microgram(s)
µl	Microlitre(s)
µM	Micromolar(s)

CHAPTER 1

INTRODUCTION

1.1 Background

The global human population is constantly growing and therefore resulting in increased consumption of food. Bread wheat (*Triticum aestivum* L.) is an important crop cultivated in the world, ranking third after maize and rice (Food and Agriculture Organization-FAO, 2016). It is, therefore, the most important staple food for most of the world's population. Globally, the production of wheat was estimated at over 680 million tonnes per year planted on about 225 million hectares (Sharma *et al*., 2013). However, the demand is expected to increase to about 813 million tonnes (20%) in 2030 and to more than 900 million tonnes (32%) in 2050 (Alexandratos *et al*., 2006). This is due to global population which is estimated to exceed 9 billion by 2050 (Roser, 2013).

On the list of most grown cereals in South Africa (SA), wheat ranks second after maize, in terms of dietary intake, the area planted and production (FAO Statistics-FAOSTAT, 2016). Wheat is undoubtedly economically important due to its contribution to human and animal diets. However, the production levels of wheat have been inconsistent resulting in the country to depending on imports (FAOSTAT, 2016). These fluctuations has reduced the market price of wheat grain, making farmers hesitant to invest in increasing wheat yield such that wheat has been out-competed by other economically important crops such as maize and soybean (United States Department of Agriculture-USDA, 2016). For example, the production increased by 31% from 1.457 million tonnes in 2015 to 1.910 million tons in 2016 (USDA, 2016), then decreased by 21% to 1.5248 million tons in 2017. Climate change, economic factors as well as different biotic factors such as insects and diseases, are the major contributors to inconsistencies in the country making it complicated to realize sustainable yields and high-quality wheat. Recent statistics of wheat production by Grain-SA (2018) are confident of the likelihood of increases in the levels of production once more to 1.808 million tonnes in 2018 season. Therefore, the crucial task researchers are faced with is advancing wheat research and coming up with novel effective and efficient approaches of increasing yields with enhanced nutritional quality of this important cereal crop.

Micronutrients are essential minerals which are required by living organisms in small quantities, these include among others: iron (Fe), zinc (Zn), boron (B), and manganese (Mn). Wheat offers over 20% of the calorie consumption particularly among low socio-economic group in developing countries (FAO, 2017). Therefore, wheat nutritional quality has an important effect on human health. Bread wheat grain is inherently deficient in micronutrients with majority of Fe and Zn mostly accumulating in the aleurone and embryo of the grain, which are separated from the endosperm during milling process (Brouns *et al*., 2012). People with wheat-based diets may suffer from dietary deficiency of important minerals such as Fe, Zn, vitamin A and iodine, hence, termed as "hidden hunger" (Kumar *et al*., 2019). Iron and Zn perform specific biochemical functions in the human body, therefore their deficiencies may damage the mental and physical growth, increase anaemia and loss of sight of children and adolescents, (Bouis and Islam, 2011). These two minerals are often lacking in the majority of rural South African diets, which makes them significant biofortification targets (Welch and Graham, 1999; Bouis and Welch, 2010).

Due to "hidden hunger", increasing micronutrient content has become a crucial breeding task. Wheat flour is currently fortified during processing with the aim to reduce the number of people with dietary deficiencies. However, implementing this strategy to be used on a large-scale is costly (Bouis and Saltzman, 2017). Alternatively increasing the density of plant minerals through biofortification presents a more cost effective strategy to reduce micronutrient deficiency (Bouis, 2003). Biofortification brings together micronutrient traits with other agronomic traits, which are required by farmers (Bouis and Saltzman, 2017). Over the past decade, a global effort using biofortification was introduced by HarvestPlus Initiative of CGIAR consortium. The program has been working on increasing the micronutrient content of staple cereal crops and has made a significant amount of progress so far (HarvestPlus, 2013).

Genetic biofortification requires using both traditional and molecular breeding approaches to characterise germplasm for mineral variability and gene-based marker assisted breeding (White and Broadley, 2011). Therefore, a broad exploration of existing genetic variations and understanding the physiology of mineral uptake, transportation and deposition in the grain, as well as the genetic sources underlying the micronutrient accumulation in grains are fundamental. Micronutrient improvement is a complicated process and has many bottlenecks including limited genetic variability of micronutrient densities in available germplasm. This is delaying advancements in breeding programs. Iron and Zn concentrations are polygenic traits that are quantitatively inherited and are strongly affected by genetic-by-environment interactions. Grain Zn is mainly controlled by

additive gene action, while grain Fe concentration is controlled by non-additive and additive gene actions (Kumar *et al*., 2013). Most of the genetic studies have so far been successful with mapping of micronutrient and yield quantitative trait loci (QTL) using linkage mapping around the world.

Linkage mapping is typically performed in segregating mapping populations developed from bi-parental crosses. These populations are inbred lines such as backcrosses, recombinant inbred lines, F2-derived populations and doubled haploid line (DH) populations. Doubled haploid line populations have advantages of producing "true-breeding" lines that can be cultivated in different environments without any genetic change (Collard *et al*., 2005). Several studies were conducted for mapping of QTLs in wheat using linkage mapping around the world (Shi *et al.,* 2008; Singh *et al.,* 2010: Patil *et al.,* 2013; Ma *et al.,* 2019). However, so far, less effort has been made to explore the genomic regions controlling micronutrient variations in South African bread wheat genotypes. Hence, there is a need to explore genetic variation in South African wheat genotypes for breeding to increase wheat yields and improve nutritional quality.

The main objectives of this study were to: (i) determine grain micronutrient (Fe and Zn) content and measure yield-related traits in doubled haploid genotypes developed and used in the Agricultural Research Council-Small Grain pre-breeding programme and, (ii) genotype doubled haploid lines to identify single nucleotide polymorphism (SNP) markers associated with high micronutrients (Fe and Zn) content and correlate with yield-related traits.

1.2 Rationale of the study

The Agricultural Research Council-Small Grain (ARC-SG) initiated a pre-breeding programme that focuses on nutritional quality of South African wheat. Initially, the focus of breeding programmes in South Africa was mainly to increase wheat yields with little attention given to improving nutritional quality. However, the expected increase in human population and over-dependence on cereal diets in the developing world exerts immense pressure on wheat breeders and researchers to come up with new strategies to develop wheat cultivars with high productivity and nutritional quality. Therefore, increasing wheat yield and the mineral micronutrients in grains has become an urgent task to reduce malnutrition. To date studies on nutritional quality have not been conducted in South African bread wheat germplasm collections of ARC-SG. Therefore, this study was conducted to

evaluate the levels of Fe and Zn concentrations and to determine QTLs influencing their genetic variation. GBS based SNP markers were used to identify the source of genetic variation and performe QTL mapping for grain micronutrient concentration and yield-related traits. The ultimate goal of this study was intended to assist the South African wheat industry by contributing towards the development of new high-yielding cultivars with improved nutritional quality.

1.3 Aim and Objectives of the study

Aim:

Improvement of micronutrient concentrations and yield components traits in South African bread wheat breeding doubled haploid (DH) lines

Objectives:

The study objectives were to:

1. Determine grain micronutrient (Fe and Zn) variation in 139 Tugela-DN and Elands DH mapping populations developed from ARC-SG wheat germplasm collection in South Africa;

2. Evaluate the mapping population for yield-related traits;

3. Correlate micronutrients and yield-related traits among the genotypes;

4. Genotype doubled haploid mapping population using SNP- and SilicoDArT-GBS based markers; and

5. Determine SNP GBS-based markers associated with high micronutrients (Fe and Zn) and yield-related traits.

1.4 Dissertation Outline

The dissertation consists of five chapters. The content of each chapter is as follows:

Chapter One provides background, motivation, aims and specific objectives of the study.

Chapter Two reviews the literature applicable to the study, thereby provides valuable insight into the work done in this research field. This chapter has sections on the wheat genomics, uses, health benefits and production levels in South Africa, as well as information relating to micronutrients such as its malnutrition impact on health, mechanism of nutrient uptake and remobilisation. Strategies for improving micronutrients and breeding technologies are also reviewed.

Chapter Three describes the methodological techniques followed the study. This chapter has been divided into three different experiments; for experiment one and two, phenotypic analysis was followed by data analysis. The last experiment is the genotypic analysis, which was conducted followed by bi-parental QTL mapping for both micronutrients and yield-related traits. Different experiments contained in the chapter are clearly separated by asterisks.

Chapter Four presents the results and discussions of each experiment conducted, separately. The chapter consists of three experiments, (i) Determination of yield-related traits, (ii) Determination of grain micronutrient (Fe and Zn) content and variation in doubled haploid lines, (iii) Bi-parental QTL map construction using SNP GBS-based markers, respectively.

Chapter Five reports the final conclusions to the findings of the study, how will the study contribute to the research community, as well as limitations and recommendations for future investigations.

Chapters and different sections and experiments within chapters were separated with Asterisks.

All the references cited in this study are presented in the reference list after chapter 5 and the Harvard referencing style was used.

All appendices are positioned after references, at the end of the dissertation.

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CHAPTER 2

REVIEW OF LITERATURE

2. Chapter outline

This chapter reports on the literature reviewed for the study. It has been divided into sections that include the brief background on wheat species and their genomics; wheat uses, health benefits and side effects of wheat grain; and its production levels in South Africa; as well as information relating to micronutrients such as its malnutrition impact on health, mechanism of nutrient uptake and remobilisation. Strategies for improving micronutrients and breeding technologies were also reviewed.

2.1 Wheat

2.1.1 Brief background

Cereals are seeds that come from grasses, for instance wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), maize (*Zea mays* L.), sorghum (Sorghum bicolor), etc. that are cultivated for the edible constituents of their grains. Cereal grains are essential as they play a vital role in human diets and are therefore staple crops. They supply humans and livestock with energy, minerals and proteins (Bouis, 2003; Bouis and Welch, 2010). On-going global efforts are being employed to reduce hunger and malnutrition, therefore, cereal improvement is important, especially in developing countries. Currently, three cereal grains i.e. wheat, rice and maize, offers the daily energy consumption of approximately 60% for the world's population (Shewry and Hey, 2015). The demand for these edible cereals is expected to highly increase due to an estimated increase in human population of 9.8 billion by 2050 (United Nations Department of Economic and Social Affairs, 2017).

Wheat is defined as any of the cereal species from the *Triticum* genus in the grass family of monocotyledons (Poaceae – formerly known as Gramineae). Wheat production has spread around the globe and has become one of the major crops that serve as a staple food (FAO, 2015). There are different types of wheat cultivated throughout the world. Common wheat (*Triticum aestivum* L.), also known as bread wheat (Figure 2.1), contributes about 95% of the wheat grown around the globe (IWGSC, 2014). Additionally, bread wheat alone as a staple food is consumed by approximately 35% of the population (Poursarebani

et al., 2014) and therefore has the highest trading value. Durum wheat (*T. turgidum var. durum* L.) is the second most cultivated species with about 5% cultivated. Club wheat (*T. aestivum* subsp. *compactum*) is a softer type of wheat. Emmer and Spelt wheat species survive as relict crops in some parts of the world, and they have found niche markets as "healthy food" (Shewry and Hey, 2015).

Figure 2.1 Wheat heads and grains

2.1.2 Genomics of *T. aestivum* L.

Wheat was domesticated more than 10 decades ago as part of the "Neolithic Revolution" when man started moving towards agricultural practices (Dubcovsky and Dvorak, 2007). The genetics of wheat are more complex than that of most other domesticated species. Wheat species differ in their ploidisation, for instance, there are diploid (two sets of chromosomes) and polyploids (four of six sets of chromosomes. *T. aestivum* L. is an allohexaploid wheat that consists of three distinct but genetically related A, B and D genomes and each with seven chromosomes (Dubcovsky and Dvorak, 2007). Bread wheat emerged because of two polyploidization events (Dubcovsky and Dvorak, 2007; IWGSC, 2014; Fig 2.2). The first event is believed to have originated when the tetraploid emmer wheat (*T. turgidum*) (AABB genome) was domesticated from the *T. urartu*, an AA-genome species and an SS-genome species, *Aegilops speltoides*. The second hybridization event was between *T. turgidum* with the AABB genome, as well as the wild diploid goat grass (*Aegilops tauschii*) a DD-genome species, which produced *Triticum aestivum* (AABBDD) (Fig. 2.2, D) (Sang, 2009; IWGSC, 2014). The hybridization process that resulted in the production of *T. aestivum* wheat has brought about its adaptability to various environments.

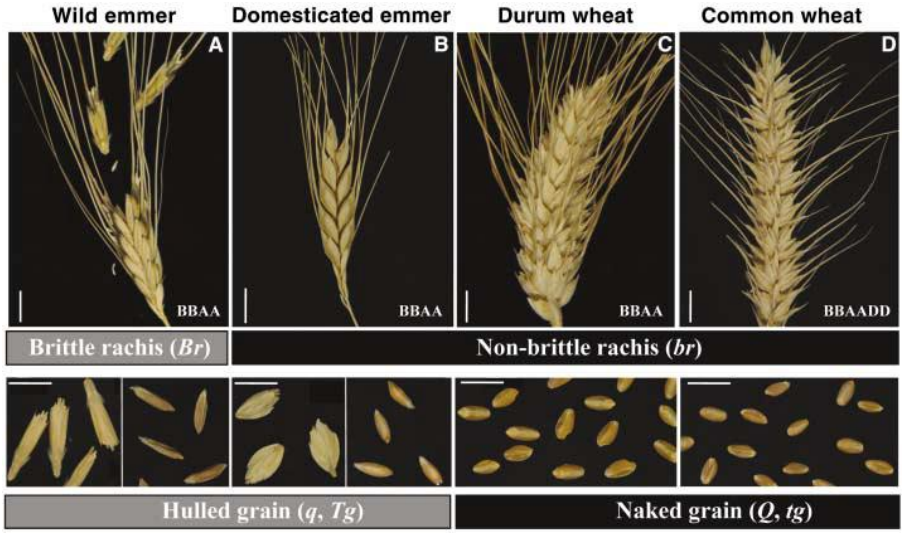

Figure 2.2 Hybridization of wheat species (Dubcovsky and Dvorak, 2007, picture by Cristobal Uauy.)

T. aestivum is a difficult crop to study because it has a large genome size of ~17 Giga-base pairs (Gbp) and is composed of ~80% repetitive sequences (Brenchley *et al*., 2012). In comparison to other grain cereals, the wheat genome surpasses maize (*Zea mays* L.) genome, which is ~2.3 giga-base pairs (Gbp), eight times (Schnable *et al.*, 2009). Wheat genome is 40 times bigger than that of the rice (*Oryza sativa* L.) genome [(~430 mega-base pairs (Mbp)] (International Rice Genome Sequencing Project, 2005). Challenges have been experienced in the past with wheat due to its large genome, thus limiting the understanding of the functions of many genes influencing significant traits. However, Brenchley *et al*. (2012) and IWGSC, (2014) have sequenced the hexaploid wheat genome using 454-pyrosequencing and identified 96 000 genes. This has paved a way for wheat genomic studies aimed at understanding the most important traits such as yield and yield components, nutritional quality and disease resistance.

2.1.3 The importance of wheat grain

Wheat is widely consumed by humans around the globe. It has key characteristics that allows predominant use for the production and processing into a variety of products. Common wheat is mainly utilised in the flour industry to produce different kinds of bread. Durum wheat is utilised for the production pasta (macaroni and spaghetti) and semolina (couscous). Club wheat is used for cakes, biscuits, crackers, pastries, and flours (Curtis, 2010). Wheat is not only restricted to the use of food products because some wheat

varieties are utilised for the production of commodities such as malt, gluten, starch (as a thickening agent), even animal feed and many more products (Shewry, 2009).

Wheat contributes towards a healthy diet as an important source of energy (carbohydrate), proteins and fibre, and to a lesser extent, vitamins, lipids, minerals and phytochemicals (Shewry and Hey, 2015). Moreover, wheat is rich in phytonutrients and thus has many health benefits. Benefits include prevention of diseases such as cancer (e.g. breast cancer), coronary heart disease, cardiovascular diseases, diabetes, constipation, irritable bowel syndrome, gastrointestinal disorders, and gallstones; wheat can also control obesity and lower blood pressure (Constabile *et al*., 2008; Anderson *et al*., 2009; Fardet, 2010; Stevenson *et al*., 2012; Shewry and Hey, 2015). Bunyavanich *et al*. (2014) reported wheat to contribute in lowering childhood asthma. Though wheat may have many health benefits, the grain residue (bran fraction) of wheat, which is rich in nutrients, minerals, etc. is used as animal feed, and therefore most of the health benefits are lost for human consumption.

Wheat is also rich in gluten proteins and starch, which makes it suitable for food and non-food products. However, it is listed among the “big eight” food allergies, with reports for both respiratory and food allergies (Shewry, 2009). Some people have allergies towards these proteins and therefore, wheat is not suitable for their health (Food Allergy Research and Education, 2016). Studies have revealed that two of the most important occupational allergies were baker’s asthma (respiratory allergy) and coeliac disease. In the United Kingdom (McDonald *et al*., 2000) and Norway (Leira *et al.,* 2005) exposure to flour dust was reported to be the second major source of occupational asthma. Coeliac disease is a chronic inflammation of the bowel, which results in the malabsorption of nutrients. The major contributing component of celiac disease is the gliadin fraction of gluten and wheat is rich in gluten (Hidalgo and Brandolini, 2014). A gluten-free diet is not easy to follow, partly because wheat gluten is added to many processed food products because of its viscoelastic properties. Consequently, studies are underway to develop gluten-free wheat or wheat with safe gluten, where the wheat gliadin genes are either modified or the immunogenic epitopes are removed using CRISPR/Cas9 (Jouanin *et al.* 2018). However, the research is still far from declaring the edited wheat crops safe for consumption, but this discovery shows the potential of genome editing in combating allergies/diseases.

2.1.4 Production of bread wheat (*T. aestivum* L.) in South Africa

The demand for increased wheat production in the world is estimated to rise by more than 60% by 2050, due to predicted increase in human population (Tilman *et al*., 2002; Alexandratos and Bruinsma, 2012). To achieve this goal, the annual yield gain of wheat productions must increase from the current level of less than 1% to at least 1.7%, annually (http://iwyp.org/). In South Africa (SA), wheat is an important cereal ranking second after maize in terms of the area planted and production. During the period from the mid-1970s to the 1980s, SA farmers sometimes produced high quantities of wheat resulting in exports that were greater than imports and the quality of this wheat was excellent (FAOSTAT, 2019). However, the current production levels are inadequate to meet the domestic demands. Therefore, SA is a net wheat importer from countries such as Argentina, USA, Germany, Canada and Russia, among others, and approximately 933 735 tonnes was imported during the 2016/17 season (Department of Agriculture, Forestry and Fisheries-DAFF, 2016). Currently, the country is reliant on the global availability of imports and the market in which wheat of good quality is expensive. During 2008/09 when there was an international crisis, several countries stopped their wheat exports, which led to the commodity becoming scarce and expensive. Should the history repeat itself, SA without its own wheat industry would be at risk of losing a large portion of its food security. Strategies such as the improvement of high yielding cultivars, cultivars that are adapted to changing climatic conditions, etc. need to be implemented for the country to rely less on imports and for farmers to continue gaining profits.

Several factors have played a major role to the challenges the country is currently facing. Factors such as the climate change, economic factors, biotic and abiotic factors and the availability of alternative crops contribute to the declining wheat production in the country. Climate change plays a major role as most of SA wheat producing areas suffer severe drought and heat stress that are induced by the recent El Niño phenomenon. This has led to the cultivation of alternative crops (e.g. soybean) with high profit potential, which were introduced to the market, replacing wheat on many farms. Furthermore, the winter rainfall in SA has been estimated to decrease in the future and this would possibly intensify the inevitable dry spells occurring during the growing season (Bradley *et al*., 2012). Moreover, due to heat stress worsening, it might therefore be almost impossible to plant winter wheat cultivars in the future as they require vernalisation to flower. The rand/dollar exchange rate also contributes to the decline in wheat production, this results in decline in the market price of wheat and the increase in the cost of post-harvest logistical services. Additionally, the implementation of open market policy in SA which allows for the

importation of cheap subsidized wheat has lowered the market price of wheat (Bester, 2014).

A detrimental abiotic factor called pre-harvest sprouting (PHS) negatively affects wheat quality (Jaiswal *et al*., 2012). Furthermore, various biotic factors contribute to production losses, and these factors include weeds, which have developed resistance towards herbicides (Heap, 2014). Diseases such as fungal rusts [yellow rust (*Puccinia striiformis f.sp. tritici* Eriks), stem rust (*P. graminis* Pers. f. sp. *tritici.* Eriks. & E.Henn), leaf rust (*Puccinia triticina* Eriks)], Fusarium Head Blight (*Fusarium graminearum*); and insect pest such as Russian Wheat Aphid (*Diuraphis noxia*) causes substantial yield losses. The abiotic and biotic factors affect wheat plants throughout or at different developmental stages, resulting in extra costs for farmers. Hence, appropriate management, such as ensuring sufficient moisture in the soil and minerals, controlling weeds, diseases and insect pests, etc., is a requirement and is crucial throughout the plant's growth.

Understanding the developmental stages of wheat is very important for biologists as yield improvement is a continuous attempt in wheat breeding. The developmental stages overlap to some degree for different yield components in their respective effect on potential grain yield (Figure 2.3). These stages play major roles in different yield components and they contribute to the overall yield (Zadoks, *et al*., 1974). However, tillering plays an important role in wheat development and the overall weight per grain, because it may considerably or completely make up for the differences in plant number after crop development, resulting in crop recovery from plant stresses (Acevedo *et al*., 2002).

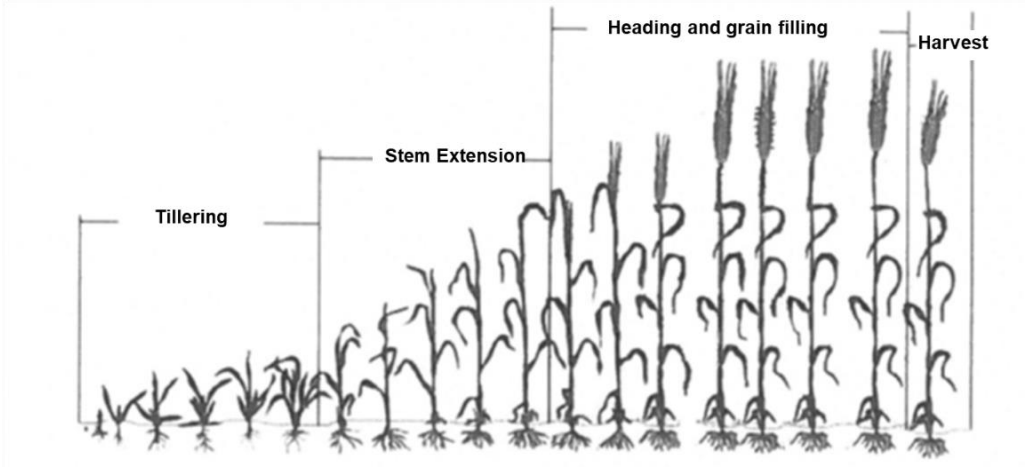

Figure 2.3 Growth and developmental stages of wheat during the growing season (ARC, 2018)

Yield-related traits such as spike number per unit area, kernel number per spike, thousand kernel weight, and seed size of wheat grain determine yield. For example, seed size has a direct linear relationship with the grain weight and it influences the milling performance, therefore the seed size is one of the important quality traits for farmers as it determines the market value of the harvest. Consequently, the seed size affects growth, yield and possibly the mineral content of the grain, and it is influenced by all the growth stages throughout the growth of the plant (Gegas *et al*., 2010; Shi *et al*., 2017).

Wheat was identified by World Health Organization (WHO) as one of the six crops to focus on in terms of improving nutritional quality and eradicating “hidden hunger” due to its health benefits. Additionally, WHO acknowledged Fe and Zn as two of the three deficient micronutrients (namely Fe, Zn, and vitamin A) important to be improved in staple crops (Ortiz-Monasterio *et al.,* 2007). Lack of these micronutrients has detrimental effects where over three billion people are micronutrient malnourished especially in developing countries (FAO, 2015). Consequently, the development of cultivars with enhanced concentrations of Fe and Zn would have an important effect on human well-being (Peleg *et al*., 2008).

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2.2 Micronutrients

2.2.1 Brief background

Cereal-based foods are among the most important staple foods and they form the biggest part of the daily diets in the world. Cereals in general have low amounts of micronutrients to meet human’s daily dietary intake. Micronutrients, for instance vitamin A, iron (Fe), zinc (Zn), boron (B), manganese (Mn), etc., are essential elements that are needed in lesser quantities for the normal growth and development of living organisms. Of particular importance is the Vitamin A, Fe and Zn, which have been selected by HarvestPlus as the three most important minerals to improve for human health. HarvestPlus is a global non-profit agricultural research program founded by Dr Howarth E. Bouis. In recent decades, soil micronutrients have slowly been exhausted by constant growth of crop yields, particularly through the Green Revolution. As a result, up to 50% and 30% of the world soil farmed for wheat is deficient in Zn and Fe, respectively (Graham and Welch, 1996; Cakmak and Kutman, 2018). This has a great impact on the amount of micronutrients available for absorption by crops. Furthermore, micronutrient deficiencies in crops result in decreased crop yields (Cakmak, 2008).

2.2.2 Impact of Fe and Zn malnutrition on human health

Genetically, modern wheat varieties have limited variation for bioavailable micronutrients (Vitamin A, Fe and Zn). This may be due to low concentrations in the soil and also the presence of inhibitors such as phytic acid and polyphenols that bind to these essential minerals (Bouis and Welch, 2010; Ramzani *et al*., 2016). Micronutrient deficiencies (hidden hunger) in humans are the results of insufficient consumption and absorption of minerals and lack of diversity of food to maintain good health and development (Bouis and Saltzman, 2017). People in developing countries, particularly in rural areas, with diets that mainly consist of cereals fall victims because the concentrations of minerals are higher in the outer parts of the grain (bran - pericarp, testa, aleurone layer; and germ/embryo; Fig. 2.4) and these tissues are lost during the milling process (Persson *et al*., 2016).

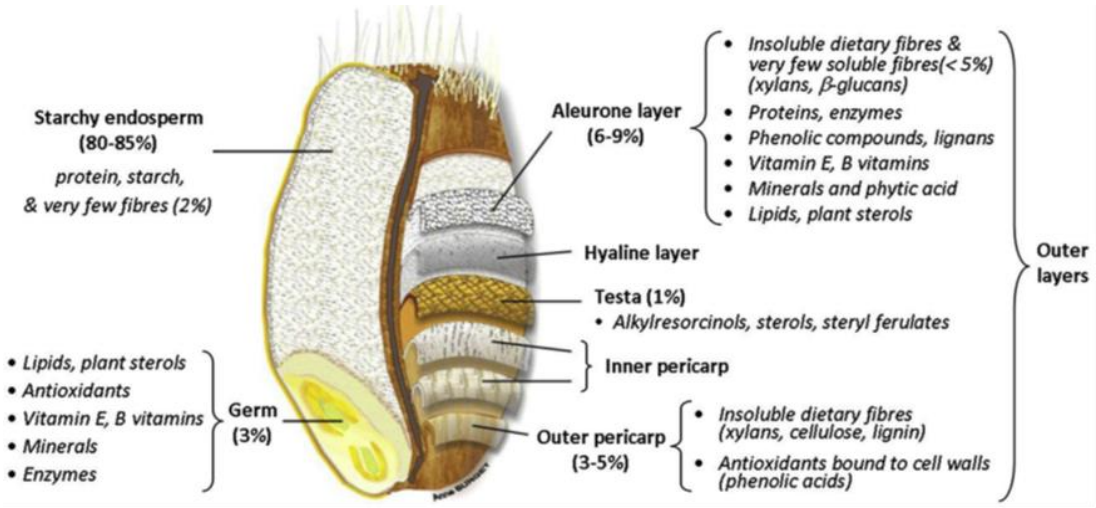

Figure 2.4 Histological structure of wheat grain (Brouns *et al*., 2012).

In developing countries where rates of mortality are high, Zn and Fe deficiency are ranked fifth and sixth among the risk factors for death and disability, respectively (WHO, 2002). About 25% of the world's population, particularly infants, pre-school children, adolescents and women of child bearing age are at most risk of being affected by anaemia linked to Fe deficiency (Velu *et al*., 2014; Gernand *et al*., 2016). Hidden hunger can lead to blindness, slow intellectual growth in children, sicknesses, susceptibility to infection, and even premature death (von Grebmer *et al*., 2014). Krężel and Maret (2016) reported an estimate of approximately 10% of all proteins in the human body are Zn-dependent. Therefore, Zn is required for growth and repair of the body tissues, healing wounds and

sound foetal development. Micronutrient malnutrition has led to the introduction of many programs to prevent and treat Fe and Zn deficiency through supplementation, ordinary fortification and biofortification of staple foods (Sharma *et al*., 2017). Currently, food fortification and biofortification strategies are most effective in combating malnutrition in poor communities where there is limited variety of food.

2.2.3 Iron and Zn variation in wheat grains

Wheat germplasm from diverse origins have been investigated regarding the Fe and Zn concentrations in the whole grain and interactions of the environment on their concentrations. Velu *et al*. (2011) discovered the existence of large variability for Fe and Zn concentrations. Another study among 81 bread wheat cultivars in Iran, showed the average concentration range of grain Fe and Zn was from 41.4-67.7 mg/kg and 36.4-73.8 mg/kg, respectively (Badakhshan *et al*., 2013). Amiri *et al*. (2015) studied 80 Iranian wheat genotypes and found the concentrations of grain Fe and Zn ranges of 63.56-102.19 mg/kg and 31.65-54.06 mg/kg, respectively. The studies revealed genetic diversities that can be utilised to improve Fe and Zn compositions using both conventional and modern breeding practices (Rawat *et al*., 2013). Furthermore, these studies illustrated the importance of considering environmental factors, for instance soil nutrient compositions and the influence they have on the mineral translocation and content of plants; and understanding the mechanisms involved in mineral translocation in the plant.

2.2.4 Understanding mechanism of nutrient uptake – from root to seed

It is important to understand the mechanism of mineral uptake in cereal grains in order to improve their content. The process of obtaining micronutrients in the grains of cereals depends on several channels that are controlled by many genes. Firstly, micronutrients are absorbed from the soil rhizosphere into the roots; translocated from roots into the plant vegetative tissues; remobilised from vegetative tissues; and finally, deposited in bioavailable forms in the seeds (Bouis and Welch 2010). The monocots like cereal grains use the chelation-based strategy to acquire micronutrients from the rhizosphere. This strategy is activated by the deficiency of micronutrients in the plant organs (López-Arredondo *et al*., 2013). Two processes are involved in the uptake of Fe and Zn from the soil, such as either (i) direct uptake of Fe^{+} and Zn^{+} by transporters [zinc regulated

transporter (*ZRT*), iron regulated transporter-like proteins (*IRT*) and 'ZRT, IRT-like Proteins' (*ZIPs*)] or (ii) via secretion of mugineic acid, which is a family of phytosiderophores. Phytosiderophore molecules form complexes with, and chelates Fe and Zn cations and are taken up by yellow stripe-like (*YSL*) transporters from the soil into the roots (Sperotto *et al*., 2014). In the roots, there is a metal chelator called nicotiamine, which is involved in transporting Fe and Zn through the roots into the vacuoles, where they are loaded into the xylem apoplastically (Lee *et al*., 2012). The micronutrients are transported into xylem where Fe chelates with citrate and Zn either chelates with citrate or moves as a cation. The transfer of micronutrients from xylem to phloem occurs in the basal part of the shoot or during remobilization from leaves during grain filling.

2.2.5 Micronutrient remobilisation and retranslocation – from leaves to seeds

The amount of micronutrients absorbed by the roots and taken up during grain filling and the amount remobilised during leaf senescence determine the quantity and concentration of nutrients in grains (Distelfeld *et al*., 2014). During leaf senescence, proteins are degraded and nutrients accumulated in the vegetative tissues are remobilised to other organs, especially the developing grains. This process permits plants to employ nutrients that are deposited in leaves during the stage when plants are photosynthetically active (Gregersen *et al*., 2008). According to Waters *et al*. (2009), when Fe and Zn are limited from the hydroponic solution post-anthesis, remobilisation of these micronutrients increases, suggesting that under nutrient limitations remobilisation might be upregulated. During senescence many genes are up-regulated in the flag leaves (Gregersen *et al*., 2008). Importantly, retranslocation of nutrients in grains is co-ordinated with leaf senescence, as a result improving the sink strength of growing grains (Uauy, *et al*., 2006b; Waters and Sankaran, 2011). Remobilisation from the vegetative tissues represents the major source of nutrients stored in the grains. For instance, in small grain cereals such as wheat, rice and barley, about 90% of the nitrogen was estimated to be remobilised from the vegetative tissues of the plant to the grains (Uauy *et* al., 2006b; Gregersen *et al*., 2008). Unfortunately, less is known about the remobilisation of micronutrients associated with senescence.

One major breakthrough that has paved a way in understanding the association between senescence process and mineral concentrations was the discovery of the wheat *Gpc-B1* locus in the wild emmer wheat. The *Gpc-B1* locus is linked to higher protein concentrations (Joppa *et al*., 1997; Olmos *et al*., 2003) and of Fe, Zn and protein

concentrations in wheat grains (Cakmak *et al*., 2004; Distelfeld *et al*., 2007). Fascinatingly, this locus is also associated with speeding the senescence process in flag leaves and with a decreased grain-filling period (Uauy *et al*., 2006a). Based on these observations, it is evident that the early senescence conferred by the *Gpc-B1* locus enhanced remobilisation of Zn, Fe and nitrogen from leaves to the grains. The results revealed that *NAM-B1* gene, which encodes the NAC transcriptional factor may play an important part in senescence and nutritional mobilisation. When the expression of *NAM-B1* in hexaploid wheat was suppressed by the RNA interference, the senescence of leaves in the whole plant was delayed and grain Fe, Zn and protein contents were decreased (Uauy *et al*., 2006b; Waters *et al*., 2009). Together these results show that senescence plays a significant role on the remobilization and retranslocation of Fe, Zn and protein, i.e. they are interconnected. Therefore, factors that affect the timing of leaf senescence could possibly influence the quantity of micronutrients that accumulate in grains.

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2.3 Biofortification

2.3.1 Brief background

Biofortification refers to an intervention strategy in which the bioavailability and concentration of nutrients in food crops are increased through agronomic practices, conventional plant breeding (White and Broadley, 2005), and modern biotechnology (Zimmermann and Hurrell, 2002) while crops are still growing. This strategy was established and executed by HarvestPlus project, which is part of the Consultative Group on International Agricultural Research (CGIAR) consortium (http://www.harvestplus.org) to increase minerals and vitamin A levels in crops consumed by resources-poor nations (HarvestPlus, 2013; Table 2.1). Biofortification aims to enhance the density of nutritional quality of plants while they are growing, as compared to supplementing nutrients when processing crops into food products (Manwaring *et al*., 2016; De Steur *et al*., 2017).

The biofortification strategy strives for an integration of micronutrient-rich trait, through plant biotechnology, in cultivars that already have desired agronomic traits, for instance high yield and resistance to diseases (Bouis and Welch, 2010). It seeks to improve food security, productivity, and the value of life and to reduce rates of mortality and morbidity associated to micronutrient malnourishment in developing countries (Sharma *et al*., 2017).

2.3.2 Strategies for biofortification of wheat

Different strategies are currently being used to improve micronutrient intake in the diet, including food fortification, supplementation and dietary diversification. Supplementation and dietary diversification are considered expensive and not economically practical to be employed on a large scale in developing countries, especially for the resource-poor people because these strategies require recurring investments (Lephuthing *et al*., 2017). A single intervention, on its own, will not improve micronutrient deficiencies, therefore biofortification was introduced to complement food fortification.

Biofortification may not provide equally high quantities of minerals and vitamins per day, in comparison to fortified food products, however it can improve micronutrient consumption for the resource-poor people who consume them regularly, in the long-term (Bouis *et al*., 2011). The levels of some minerals have already been improved by HarvestPlus in various staple foods such as, wheat, rice, cassava, maize, pearl millet, bean and sweet potato (Table 2.1). A prerequisite for biofortification is the exploitation of existing genetic variations. In crops where the target nutrient does not naturally exist at required levels, transgenic plant breeding has proven to be an approach with promising potential to supply biofortified crops with required nutrients without compromising yield. Trijatmiko *et al*. (2016) conducted a study and developed transgenic Fe and Zn rich rice (golden rice), tested it in confined field trials and achieved 30% of expected average requirements (EAR) in the human diet for both nutrients. Golden rice with high level of beta carotene, can supply more than 50% of the EAR for vitamin A. However, the production of golden rice has not been established in any country due to regulatory approval processes (Wesseler and Zilberman, 2014).

Transgenic breeding has been adopted by many countries including the Unites States, Nigeria, European countries, Brazil, Argentina, among others (de Steur *et al*., 2015), for improving crops using genetic modification (GM). Different crops such as soybean, maize, cotton, cowpea, canola, and many more have been modified mainly for resistance to diseases and herbicide tolerance (Crop Biotech, 2019). A review was conducted between 1995 and 2014 for three major GM crops: soybean, maize and cotton. According to Qaim and Klümper (2014) farmers who adopt GM crops attained a 69% increase of profits above farmers who did not. A new transgenic-based approach that has been introduced recently is gene editing.

Table 2.1 Biofortified crops released by HarvestPlus (Adapted from Bouis *et al*., 2011)

Crop	Nutrient	Countries of first release	Agronomic trait	Year of release
Sweet potato	Vitamin A	Uganda, Mozambique	Disease resistance, drought and acid soil tolerance	2007
Bean	Iron, Zinc	Rwanda, DR of Congo	Virus resistance, heat and drought tolerance	2012
Pearl Millet	Iron, Zinc	India	Mildew resistance and drought tolerance	2012
Cassava	Vitamin A	Nigeria, DR of Congo	Disease resistance	2011, 2014
Rice	Zinc, Iron	Bangladesh, India	Disease and pest resistance, cold and submergence tolerance	2013
Wheat	Zinc, Iron	India, Pakistan	Disease and lodging resistance	2013
Maize	Vitamin A	Zambia, Nigeria	Disease resistance and drought tolerance	2012, 2015

This approach may possibly be used to improve wheat production, quality and resistance against biotic and abiotic stresses. One ground-breaking technology for genome editing was the use of CRISPR/Cas9 (Zhang *et al*., 2016a). The main advantage of this technology is to provide an opportunity for targeting multiple sites simultaneously and therefore can be used to speed up plant breeding to combat the crisis of food security and malnutrition (Cao *et al*., 2016). Although, transgenic crops may deliver remarkably high levels of nutrition, their distribution to farmers and production for human consumption depends on the approval by the national biosafety and regulatory processes (Bouis and Saltzman, 2017). A study by McPhetres *et al*. (2019) documented that spreading the knowledge to people would possibly teach them about the science behind GM foods and thus they might develop positive attitudes towards it and a greater willingness to consume GM foods.

2.3.3 The challenges of biofortification

Advances in micronutrients enhancement through the improvement of genetic pathways are limited and this is due to the unfortunate fact that heavy metals such as cadmium (Cd) use the similar mineral transporters as Fe and Zn (Zhao and McGrath, 2009; Manwaring *et al*., 2016). Cadmium is a non-specific metal, which is toxic for human

consumption. *ZIP* family protein transporters, such as *IRT1* and *IRT2*, are the major ferrous ion (Fe^{2+}) uptake proteins in *Arabidopsis thaliana* root cells and *IRT1* also improves the absorption of Zn and Cd (Guerinot, 2000). Studies have examined the potential disadvantages of biofortification, e.g. through the improvement of *ZIP*s and found that by improving Zn absorption by the roots, *ZIP*s can also improve the uptake of Cd. Arduini *et al*. (2014) reported high amounts of Cd concentrations in durum wheat varieties grown in Italy, these amounts exceeded the acceptable limit of 0.2 mg Cd/kg. Therefore, if biofortification is used to improve this pathway, this could lead to an unwanted detrimental effect of making plants poisonous and dangerous for human consumption (Manwaring *et al*., 2016). In contrast, Greger *et al*. (2016) detected that Silicon (Si) decreased Cd content in wheat grains and shoot, and consequently Si upregulated *IRT1* in root and shoot improved Fe translocation in wheat. Silicon was found to bind to cell walls locking-in Cd, thus inhibiting the cellular uptake of Cd (Liu *et al*., 2013). Therefore, this indicates that improving pathways could be a possible solution to increase micronutrient concentration while many challenges on the safety of the biofortification of food remains.

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2.4 Sequencing

2.4.1 Next generation sequencing

Next generation sequencing (NGS) is the term used to define many different sequencing technologies such as, genotyping-by-sequence (GBS), Roche 454 sequencing, Illumina (Solexa) sequencing, Ion torrent: Proton/PGM sequencing and SOLiD sequencing (Luo *et al*., 2012). These technologies have granted scientists the opportunity to sequence larger DNA and RNA strands more rapid and cheaper because they have longer read length and higher accuracy. The introduction of NGS has brought a revolution in the sequencing of the whole genome of various plant species such as *Arabidopsis thaliana* (Arabidopsis Genome Initiative, 2000), rice (Yu *et al*., 2002) and barley (Mayer *et al*., 2012).

T. aestivum L., with its gigantic allohexaploid genome that is complex and has high repeat content, presented challenges for the whole genome to be analysed (Khan and Budak, 2015). In 2005, the IWGSC started the initiative using molecular breeding with the aim to provide a basis for improvement of and to provide an annotated reference genome sequence of excellent quality for bread wheat. Since then, a lot of efforts have been made to sequence *T. aestivum*, e.g. Brenchley *et al*. (2012) reported on sequencing the wheat

variety, Chinese Spring (CS42), using the Roche 454-pyrosequencing technology. Between 94 000 and 96 000 genes were discovered and two-thirds were allocated to the A, B and D genomes of wheat. Moreover, IWGSC (2016) identified 97% of genes that were assigned to 21 wheat chromosomes based on the Illumina sequencing data assembled with NRGene's DeNovoMAGICTMTM software, providing a technology for advancing gene discovery and improving this major crop. Recently, IWGSC (2018) "finally cracked the code", providing an interpreted reference sequence representing 21 chromosome-like sequence assemblies. With this breakthrough, IWGSC have identified 107 891 high-confidence genes as well as their genomic background of regulatory sequences. This annotated reference sequence has formed the basis for advancing wheat research and application resulting in a better knowledge of wheat biology and genomics-assisted breeding. These developments in sequencing procedures, have reduced the costs of DNA sequencing thus allowing for genotyping-by-sequencing techniques to be possible for species with complex large genomes.

2.4.2 Genotyping-by-sequencing

Genotyping-by-sequence (GBS) is a more efficient and cost-effective approach that was developed by Elshire *et al*. (2011). It is a promising genomic approach that has recently emerged for investigating diversity within the plant genetic on a genome-wide scale and it offers a quicker and high-throughput means to analyse different genome species on a wide scale (Poland and Rife, 2012; Peterson *et al*., 2014). Genotyping-by-sequence targets the genomic sequence closest to the restriction enzyme locations to generate a condensed representation of the genome (Poland *et al*., 2012). Due to this, GBS has the ability to determine and detect large numbers of single nucleotide polymorphisms (SNPs) and it is capable to evaluate large sets of markers used in genetic analysis. A highly multiplexed GBS system was created for the development of short libraries to be utilised in the Illumina NGS platform (Elshire *et al*., 2011). Their results on maize and barley suggested that future application of GBS may allow plant breeders and conservation biologists the ability to perform genomic selections on novel germplasm and species not previously studied without the need to first develop any molecular tools. Thus, GBS technology proved to be a valuable method for genetic mapping in other species, such as rice (Huang *et al*., 2009), sorghum (Morris *et al*., 2013), wheat (Poland *et al*., 2012), soybean (Jarquin *et al*., 2014) and maize (Zhang *et al*., 2015a). Understanding the importance of GBS as a genotyping tool of NGS world, it was used for variation analysis and QTL mapping in the present study.

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2.5 Mapping populations, molecular genetic markers and Quantitative trait loci analysis

Quantitative traits (also known as polygenic traits) are traits that are controlled by many genes of large effect, small effect or a combination of both (Collard *et al*., 2005). Many traits of importance in agriculture, for example yield, quality and other disease resistances are polygenic traits and can also be influenced by the environment (Collard *et al*., 2005; Velu and Singh, 2012; Mondal *et al*., 2016). Regions in the genome that contain genes or are tightly linked to genes affecting certain quantitative traits are known as QTL. The process of performing linkage analysis, to detect QTL related with traits, and therefore creating linkage maps is referred to as a QTL mapping. Scientists use QTL mapping not only to determine genes responsible for important traits but also to investigate the influence of the environment and G x E interaction on phenotypes. However, the major challenge is understanding the influence of genes and environment on quantitative phenotypes.

Linkage mapping studies are performed in segregating mapping populations that are developed from crosses between lines/genotypes/cultivars that differ for important trait(s). Population types that are mostly used for QTL mapping are the parental inbred lines such as backcrosses, DH and RILs (Falconer and Mackay, 1996). Doubled haploids and RIL populations possess the advantage of producing homozygous or “true-breeding” lines that can be multiplied, replicated without the genetic structure changing and grown in many different environments (Collard *et al*., 2005). Therefore, the overdominance – that is contributed by heterozygotes, which are almost absent in inbred lines – does not affect the QTL analysis. Furthermore, the fact that at each polymorphic locus only two genotypes are possible simplifies the use of inbred lines.

Recently, plant (and animal) breeders have been using DNA markers (also known as molecular or genetic markers) to understand the genetic basis of important traits. The use of these markers has unlocked a new field in agriculture known as “molecular breeding”. Various DNA marker types are widely accessible to characterise different populations for QTL mapping, however they must be selected thoughtfully according to the goals of the project. The development of DNA markers have paved a way in the characterisation of quantitative traits. These markers include: restriction fragment length polymorphism,

cleaved amplified polymorphic sequences, randomly amplified polymorphic DNA, simple sequence repeats, single nucleotide polymorphism, expressed sequence tags and diversity arrays technology (DArT) markers (Semagn *et al*., 2006). To effectively identify QTL, the main requirement is a genetic linkage map that is densely populated with genetic markers. Genetic markers that are utilised to generate linkage maps must display high levels of polymorphism – ability to differentiate amongst individuals – between parents that possess traits of interest and are also used to score individuals' genotypes in the study population (Collard *et al*., 2005).

Knowledge of the genetic basis underlying agronomic traits and mapping of their QTL will help decipher genes influencing the inherent variation and yield improvement. Thus, facilitate the development of plant breeding approaches for enhancing grain micronutrient concentrations in high yielding varieties by means of marker-assisted selection (MAS). Previously, linkage maps were greatly reliant on morphological markers to facilitate the association of phenotypic traits to genes or regions on chromosomes. However, with current improvements in biotechnology high-density maps that include thousands of molecular markers were developed (Hyten and Lee, 2016). To date, several studies have identified genetic variation of micronutrients in the wheat grain and the correlation of these micronutrients with each other and their related yield components (Peleg *et al.,* 2009; Liu *et al*., 2014; Velu *et al*., 2017).

In the past years, many QTLs have been identified and recorded for micronutrient concentration using DH or RIL. Peleg *et al*. (2009) conducted a QTL mapping study for micronutrients and macronutrients concentration in a RIL population. The study led to the identification of 82 QTLs mapped for 10 minerals. Most QTLs (50 QTLs) were in favour of the wild allele, which has been reported to be associated with increased contents of protein, Fe and Zn and can be used for MAS breeding (Tiwari *et al*., 2010; Rawat *et al*., 2011). In addition, grain protein concentration was found significantly correlating with Fe, Zn and copper (Cu) concentrations, indicating a mutual physiological and/or genetic factors influencing these mineral concentrations. A recent study was conducted by Velu *et al*. (2017) using DArT markers on two RIL mapping populations developed from a tetraploid and a hexaploid population. They reported two QTLs (1B and 6B) for grain Zn (GZn) and one QTL on chromosome 2B for GZn which shared a marker interval with grain Fe (GFe), indicating the possibility of concurrent enhancement of GFe and GZn. Grain Zn concentration (GZnC) was determined in a RIL population and two novel QTLs with large effects were consistently identified for increases in GZnC on chromosome 2Bc (centromere region) and 3AL (long arm) (Hao *et al*., 2014). The 2Bc QTL was found to have pleiotropic

effect and increases 1000-kernel weight (TKW) at significant levels (Hao *et al*., 2014). However, Liu *et al*. (2014) reported that Fe and Zn concentrations were negatively associated with grain yield. They reported that for every 1000 kg ha^{-1} increases in grain yield, a decrease in Fe concentration by 2.1 mg/kg and 1.3 mg/kg and in Zn concentration reduced by 0.9 mg/kg and 1.3 mg/kg, for spring and winter wheat was observed, respectively. Additionally, Gorafi *et al*. (2016) reported negative associations between kernel weight and Fe and Zn. These contrasting results show the advancements in breeding for high yielding cultivars with improved nutritional qualities and major challenges that researchers are still facing due to environmental effects playing a major role in different yield components and nutritional traits. These advancements have been made possible by the platforms called sequencing technologies, which have enabled researchers to better understand the genetic mechanisms underlying these polygenic traits.

Summary of the review

This literature review determined that there is a need to increase food production for more sustainable agriculture, especially under stress conditions as the world is experiencing global warming. Therefore, adaptation strategies need to be implemented to develop improved germplasm. The expected increase in human population exerts immense pressure on wheat breeders and researchers in general to come up with new strategies to close the growing yield gap and improve food security. Although SA is regarded as a “food secure” country, more than half of the households in rural arears are still living in poverty and wheat production is declining at an increased rate, more especially in the dryland areas. If this trend continues, the food security might come under pressure. Biofortification is one of the most important approaches that breeders should invest in, as it is sustainable and cost effective in the long-term. The use of high-throughput tools such as transgenic solutions, genome editing and next generation sequencing to study genes and gene pathways that influence certain traits such as the yield components and the amounts of minerals will assist in achieving required levels/concentrations of minerals in high yielding cultivars. A multidisciplinary approach involving agronomists, breeders, geneticists, physiologists, entomologist and pathologists at different stages of research and development is necessary to develop climate resilient, high yielding and nutritious crop cultivars that are urgently needed to respond to current ecological and societal challenges.

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CHAPTER 3

METHODOLOGY

This study received ethical clearance, Appendix I [Ref no. 2018/CAES//045]

3. Chapter outline

The chapter consists of two sections. The first section provides details of the production and characteristics of doubled haploid lines as well as materials used for the study. The second section provides relevant details of the experiments, namely: (i) evaluation of yield-related traits; (ii) determination of grain micronutrient (Fe and Zn) content and variation; and lastly, bi-parental QTL map construction using SNP GBS-based markers.

3.1. The development and characteristics of doubled haploid lines

The planting material was received from the National Small Grain Germplasm Collection at Agricultural Research Council–Small Grain (ARC-SG). The planting material consisted of 139 doubled haploid (DH) lines crossed from Tugela-DN and Elands parental cultivars, together with the parental cultivars. The DH population was developed by doubling the gametes of F_1 or F_2 population. After the production of F1 or F2 populations, pollen grains or haploid embryos induced chromosome doubling. Thereafter, plants underwent regeneration using tissue culture techniques. The DH bi-parental population was developed by crossing Tugela-DN and Elands cultivars. Tugela-DN was the first winter wheat cultivar that was released for dryland production in 1992 with resistance to Russian wheat aphid biotype 1 (RWASA1) and all rust diseases (stem, leaf and stripe). It also has very good straw strength and good aluminum tolerance. Tugela-DN has low to high yield potential and good bread-making quality (ARC, 1993). Elands is a facultative cultivar released for dryland production in 1998 with resistance to RWASA1. It has medium to high yield potential, medium growth length, good straw strength, and excellent pre-harvest sprouting tolerance. Elands also has an exceptional bread-making quality and serves as a standard check for bread quality in SA (ARC, 1999).

3.2. Experimental design

The present study was conducted as three different experiments. The experiments were conducted in the greenhouse for genotyping purposes and in the field to better understand the environmental effect on micronutrients and to investigate factors influencing yield-related traits. Field experiments were conducted in 2017/18 season at Arlington, Bethlehem and Harrismith and in 2018/19 season at Bethlehem. All experimental environments were in the Free State province under rain-fed conditions and were designated ARL18, BHM18, HAR18, and BHM19, respectively. The DH lines and parental cultivars were planted using the augmented design, where only the parental cultivars were replicated four times and the DH lines were replicated once in the design. The entries were planted in a one-meter row length and spaced at 0.45 cm between the rows.

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3.3. Experiments

The present study was conducted as three different experiments.

3.3.1 Experiment I: Evaluation of yield-related traits

3.3.1.1 Determination of yield-related traits

Yield-related traits were evaluated when the plants were physiologically mature, eight spikes (heads) from each line/cultivar were harvested for evaluations and averages were used for further analysis. Kernel number per spike (KNPS), spike length (SL), grain weight per spike (GWPS), 1000 kernel weight (TKW) spikelet number per spike (SPS), and grain size (GS) were recorded in the laboratory (Figure 3.1). SL was measured at maturity starting from the bottom of the rachis up to the topmost spikelet, not including the awns and expressed in centimetres (Figure 3.1A). SPS was measured by counting the spikelets excluding the bottom and the top spikelets (Figure 3.1B). The grains were dehusked manually (hand threshed) to avoid metallic contamination or seeds from mixing, then KNPS was measured by counting the seeds (Figure 3.1C). GWPS was measured by weighing the total number of seeds per spike and was expressed in grams (Figure 3.1D). TKW was measured in grams by counting and weighing 1000 seeds per sample using a SeedCount machine (Elmor Ltd, Switzerland) (Figure 3.1E). Finally, GS was measured in millimetres from the pictures taken using SONY camera, model DSC-w5 (Sony Corporation, Kōnan, Tokyo) (Figure 3.1F) and uploaded into the SmartGrain software (Tanabata *et al*., 2012).

The SmartGrain determines grain length, width, area, circularity and perimeter length and length-to-width ratio, but in this study only results for grain length (GL) and width (GW) will be reported.

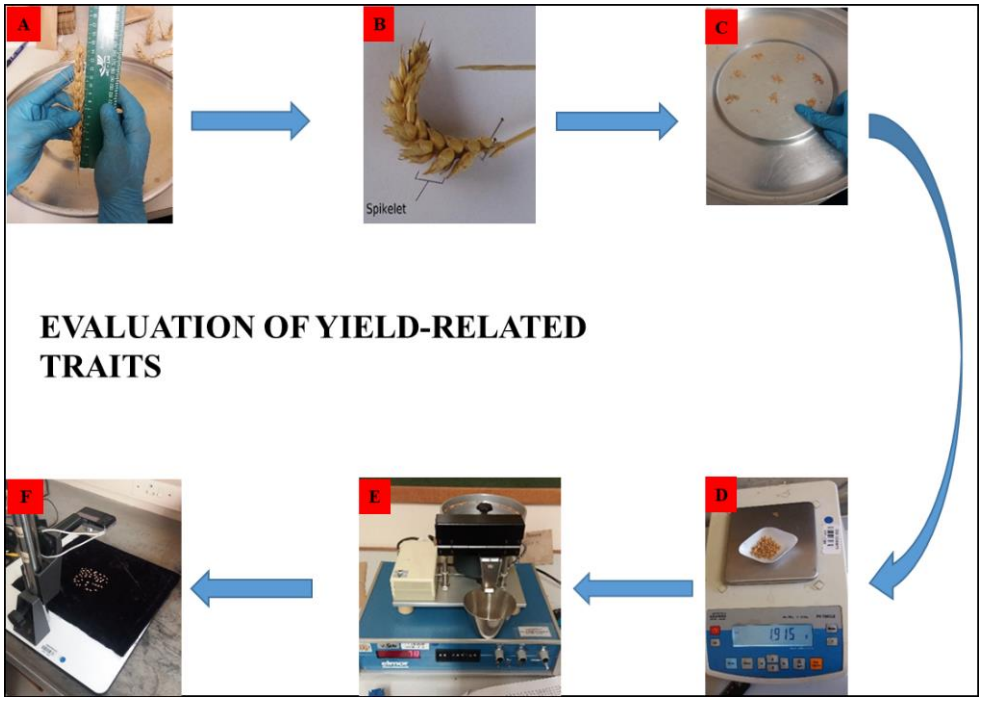

Figure 3.1 Evaluation of yield-related traits: (A) measuring spike length, (B) counting spikelets per spike, (C) counting seeds per spike after threshing manually, (D) weighing kernels per spike, (E) counting TKW per sample, and (F) camera mounted to a stand to take pictures for grain size measurements.

3.3.1.2 Statistical analysis

Yield-related traits for each cultivar and DH lines from all environments were analysed to determine frequency/normal distribution, analysis of variance (ANOVA) and correlation coefficients. Data analysis was performed using GenStat software 18th edition. A general ANOVA for parental lines was used and since DH lines were not replicated, analysis of an unbalanced design using GenStat regression was used.

3.3.1.3 Heritability

Heritability is the measure of how much variation in a trait was contributed by the genetic differences versus the environment influences. A heritability close to one specifies that the variation seen in a trait is because of the genetic differences and the environmental

factors have slight influences, and vice versa. Variance components for genotype and genotyping-by-environment interaction were used to determine the estimations of the broad-sense heritability (h_B^2) according to Tsilo *et al*. (2010) as follows:

$$h_B^2 = \frac{\sigma_g^2}{[\sigma_g^2+(\sigma_{ge}^2/e)+(\sigma_e^2/re)} \quad \text{or} \quad 1 - \frac{MSge}{MSg}$$

where MS_g and MS_{ge} represents mean squares of genotype and genotyping-by-environment, respectively; σ_g^2 represents the genotypic variance = (MS_g - MS_{ge})/(*re*), σ_{ge}^2 represents the genotyping-by-environment interaction variance = (MS_{ge} - MS_e)/*r*, σ_e^2 represents the error variance = MS_e, *r* represents the number of replications, *e* represents the number of environments.

3.3.2 Experiment II: Determination of grain micronutrient (Fe and Zn) content and variation in doubled haploid lines

3.3.2.1 Sample preparation

For micronutrient analysis, seven grams of seeds for each sample were rinsed in tap water for one minute to remove dust particles from seed surface and oven dried at 45°C for six hours. Then seeds were finely ground into flour using the POLYMIX® PX-MFC 90 D laboratory non-rust steel mill. This milling machine runs at 6 000 repeats per minute (rpm) and thus capable of grinding dry substances, such as cereals, grains, nutshells, etc. The flour samples were passed through 0.8 mm diameter sieve. Analyses were conducted at ARC-Institute for Soil, Climate and Water (ARC-SCW), Analytic Services laboratory in Pretoria, South Africa for Fe and Zn content analyses.

3.3.2.1 Digestion process and micronutrient concentration determination process

One gram of flour samples were digested using 7 ml of concentrated HNO_3 (nitric acid) and 3 ml of $NCLO_4$ (perchloric acid) at temperatures of up to 200 degrees celcius. The mineral concentration of each sample was determined from a 100 ml volume using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES) at ARC-ISCW. Iron and Zn were determined in the wavelengths 940 nm (Fe) and 856 nm (Zn) chosen for highly sensitivity and lack of spectral interferences.

3.3.2.2 Statistical analysis

Data analysis was conducted as described in section 3.3.1.2.

3.3.2.3 Heritability

Broad sense heritability was estimated as described in section 3.3.1.3.

3.3.3 Experiment III: Bi-parental Quantitative trait loci map construction using SNP GBS-based markers

3.3.3.1 Preparation of leaf samples for DNA isolation

Five seeds from each cultivar/line were planted in 2 litre pots and grown in the greenhouse, under controlled conditions. When the seedlings entered the tillering stage (21 days old, Zadoks scale 22-25) (Zadoks *et al*., 1974), three to four young leaves from each of the 139 DH lines and two parents, Tugela-DN and Elands, were harvested by cutting the leaves with a sterile scissor into 2 ml Eppendorf tubes (Figure 3.2). The harvested leaves were temporarily stored in the freezer at -80°C until DNA extraction.

3.3.3.2 DNA extraction and DArT-seq genotyping

The genomic DNA was extracted from the 139 DH lines and parental cultivars Tugela-DN and Elands using the Diversity Arrays Technology (DArT) isolation procedure (http://www.diversityarrays.com/). The quality, purity and quantity of DNA samples was tested on the NanoDrop 2000 spectrophotometer (ND-2000 V3.5, NanoDrop Technologies, Inc.). The absorbance ratio of A260/A280 was used to determine the contaminations of protein compounds and gel electrophoresis on 0.8 % (w/v) agarose gel. For genotyping, 500-1000 ng of restriction grade DNA samples, suspended in TE buffer with a final concentration of 50-100 ng/µl were shipped to Diversity Arrays Technology, Pty Ltd, Yarralumla, ACT, Australia for genome profiling using a GBS platform known as DArT-seq.

Figure 3.2 Harvesting of leaf material: (A) 21-day old seedlings; (B) Sterilising scissor with ethanol; (C) Cutting out of the leaf; (D) Leaf tissue material inserted into Eppendorf tubes; (E) Temporary storage of leaf tissue material in a cooler box

Generally, DArT-seq system generated two kinds of markers, SNP and present/absent variation, also called SilicoDArTs. For this study, SNP GBS-based markers were utilised for construction of genetic linkage map and QTL analysis. All markers that were homozygous – markers that had identical genotype in parents or both parents with missing data were deleted, redundant and non-informative markers were filtered, and SNP markers that presented multiple genetic positions were removed. Additionally, SNP markers with missing data that exceeded 10% were also deleted, resulting in 1462 out of 3204 markers used.

3.3.3.3 Construction of a genetic linkage map

All polymorphic SNP markers were converted into genotype codes (“A”, “B”) based on the scores of the parental cultivars after genotyping mapping population with DArT-Seq. A linkage map was constructed in a series of steps. In the first step, redundant markers were removed using BIN tool algorithm implemented in inclusive composite interval mapping v4.1 (IciMapping) (http://www.isbreeding.net/) software on 1 462 SNP markers. After binning, markers were grouped using logarithm of the odds (LOD) threshold value of

3.0. Genetic distances between markers were calculated based on the Kosambi function (Kosambi, 1943). Ordering within linkage groups (LGs) was conducted with nnTwoOpt function (an efficient approximate algorithm for solving traveling salesmen problems in which nearest neighbours are used for tour construction improvement). After redundant markers were removed, the initial linkage map was constructed with 986 SNP markers in R/qtl (Arends *et al*., 2010) package available in R Statistical Computing Environment (R Core Team R, 2015). The initial linkage map was used to inspect the markers and genotypes data for duplicate lines/markers, segregation distortion, switched alleles, single and double cross-overs (genotyping errors) using the appropriate functions. This step is crucial to identify and correct errors in the data.

After checking data about two-thirds of markers were deleted. In the second step, genotypic data with 318 filtered SNP markers was used for final linkage map construction in IciMapping v4.1. Linkage groups were determined with LOD threshold value of 3.0, and 21 LGs representing all 21 chromosomes were identified. The ordering of markers distributed over 21 chromosomes was performed using 'nnTwoOpt' function. The order of markers was fine-tuned with the 'rippling' function performed using the sum of adjacent recombination frequencies (SARF) with a window size of 5 as rippling criteria. Genetic distances of markers based on recombination rate were converted to centimorgans (cM) using the Kosambi mapping function (Kosambi, 1943). Finally, the best marker order with the shortest linkage map distance was selected and a QTL map was constructed.

3.3.3.4 QTL analyses

Quantitative trait loci (QTL) analyses were carried out by means of single QTL (interval mapping), two-dimensional and multiple-QTL models in the R-based software package, R/qtl (Broman and Sen, 2009). The Haley-Knott regression method (Haley and Knott, 1992) was used to perform QTL analyses, missing genotypes probabilities were calculated, and a step interval of 2 cM was used to simulate genotypes with 64 draws per genotype and presuming a genotyping error rate of 0.01. Haley-Knott regression is dependent on probabilities of genotype amongst the marker data and provides a fast approximation in estimating QTL effects and in power to detect QTL.

A series of computational steps were used to investigate the genotypic and phenotypic associations:

Step 1: The precision of the LOD scores for each phenotypic trait and the estimated significance threshold (α= 0.05) were established using 1000 permutations of both genotypic and phenotypic data (Broman and Sen, 2009).

Step 2: The 'scanone' function was used to perform single QTL analyses, where each genomic position was analysed, one at the time.

Step 3: The two-dimensional QTL model was used to test the interactions between QTLs to test the existence of epistatic interactions. The 'scantwopermhk' function was used for this purpose.

Step 4: All significant QTLs, which were detected were fitted into a multiple QTL model after calculating the genotypic probability.

Step 5: A 'QTLobject' was created using 'makeqtl' function and this specified the location of a set of putative QTLs to be considered. Finally, a function 'fitqtl' was used to estimate the percentage explained by the phenotypic variance.

A major QTL was defined as a QTL with a LOD value score >2 and a phenotypic variance contribution of ~ 5% or more in at least two of the four environments. Only those QTLs that were detected in at least two environments (out of four) or were associated with at least two traits were reported in this study. A stable QTL was defined as a QTL that showed significance in at least two environments or that was associated with at least two traits. QTLs were categorised based on PVE values, as major QTLs if they have a minimum PVE of 10 or more and minor QTLs if they have a PVE value less than 10. Few QTLs were considered to be valid, as an exception, if they were identified in only one environment but met all statistical requirements, i.e. high PVE and LOD values. The designation of a QTL name was labelled based on a set of rules according to Cui *et al*. (2012) based on a set of rules. Firstly, *italic* uppercase *'Q*' symbolizes 'QTL'. Secondly were letters that abbreviated the traits. Thirdly, the uppercase numeral letters, 'A', 'B' or 'D', specified the chromosome of wheat to specify the location of the QTL. Fourthly, after the second period, the last numeral represents the environment(s) where the QTL was identified. Lastly, in cases where two different QTLs were identified for the same trait, lowercase letters, e.g., a, b, c or d, were used to differentiate them.

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CHAPTER 4

RESULTS AND DISCUSSION

4. Chapter outline

This chapter reports on the results and discussions of each analysis. It consists of three experiments namely: (i) evaluation of yield-related traits; (ii) determination of grain micronutrient (Fe and Zn) content and variation; and lastly, bi-parental QTL map construction using SNP GBS-based markers.

4.1 Experiment I: Evaluation of yield-related traits

4.1.1 Results

Phenotypic variation of doubled haploid lines

All traits were evaluated based on averages of eight heads/spikes per sample. The frequency distribution of all seven traits over environments showed approximately normal distributions (Figure 4.1), indicating polygenic inheritance as expected for quantitative traits. Most of the DH lines outperformed the parents for all traits, suggesting transgressive segregation (Appendix III). Additionally, the results indicate the occurrence of large variation among the DHs studied.

Values of mean, ranges and standard deviations for all traits are summarised in Appendix III, together with mean values for parental lines. The mean performance of parents was higher in Bethlehem (BHM) for one season or both, except for GL where Arlington (ARL18) and Harrismith (HAR18) had higher averages (Appendix III). Elands exhibited lower means for all yield-related traits in three environments except for 1000-kernel weight (TKW), as compared to parent Tugela-DN. Doubled haploid lines had the same trend as the parents and the mean performance was higher in BHM, for one season or both, except for grain length, where ARL18 and HAR18 had higher averages of 9.8 mm. A wide variation was observed for all traits. For instance, KNPS showed a mean value of 41.2 and standard deviation of 8.3 with a minimum of 17.1 and a maximum 61.1 for samples averaged across all four environments (Appendix III). The parental cultivars had a comparably wide variation for trait values in all four test environments.

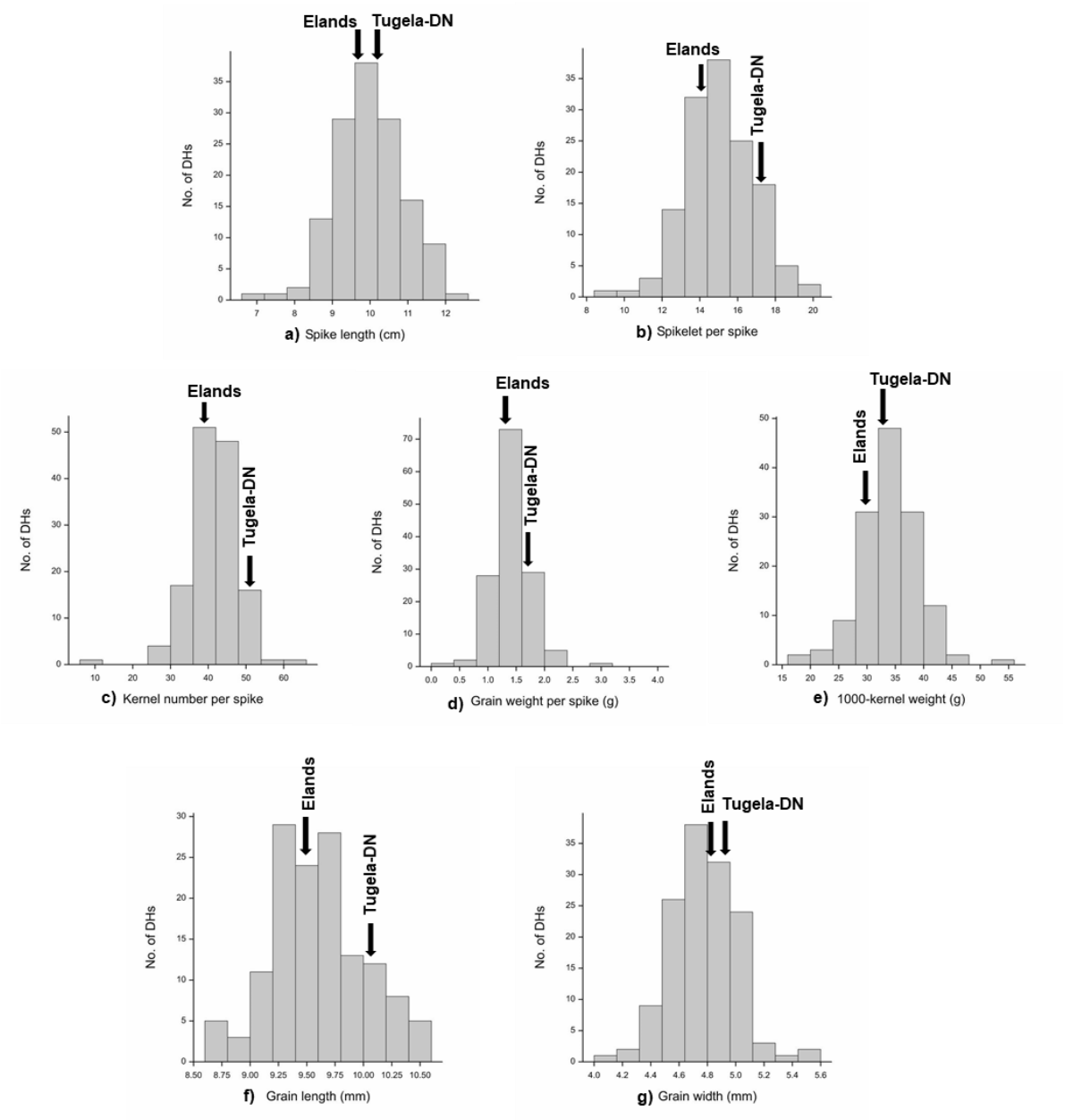

Figure 4.1 Histogram pattern of 139 DHs for four locations mean values of a) spike length, b) spikelet per spike, c) kernel number per spike, d) grain weight per spike, e) 1000-kernel weight, f) grain length, and g) grain width. Parental means are marked with *arrows*

Analysis of variance

The analysis of variance (ANOVA) indicated significant variances among genotypes (DHs) for all seven traits across the environments (Table 4.1). Both genotypic and environmental effects had significant influence on the traits. The broad-sense heritability estimates for GW,

GWPS, KNPS, TKW, SL, GL and SPS were 45%, 53%, 54%, 56%, 57%, 65% and 67%, respectively (Table 4.1).

Table 4.1 Analysis of variance (ANOVA) of genotype and environment effects and proportion of phenotypic variation of yield-related traits obtained across environments among DH lines

	Mean square				Heritability
Trait[a]	Genotype	Environment	G*E	Error[b]	
GWPS	0.289***	17.638***	0.137^{ns}	0.0972	0.53
TKW	75.36***	6730.75***	33.48^{ns}	25.73	0.56
KNPS	116.51***	4255.31***	53.61^{ns}	38.61	0.54
SL	2.456***	195.17***	1.061*	0.444	0.57
SPS	8.753***	1252.88***	2.861*	1.537	0.67
GL	0.450***	16.640***	0.1595***	0.0457	0.65
GW	0.139***	5.047***	0.076*	0.0413	0.45

[a] Traits were defined in Table 4.1

*, ** and *** denotes significance at $P < 0.05$, 0.01 and 0.001 probability levels, respectively; ns denotes non significance at $P < 0.05$

[b] Error mean squares were estimated from the check genotypes that were replicated within environments, as described in an augmented design by Federer (1961)

Correlations

Most yield-related traits were significantly correlated with each other, except grain length. Kernel number per spike (KNPS) was significantly correlated with spike length (SL) (r = 0.53) when averaged across all environments and the strongest correlation was observed in BHM18 (r = 0.66). Spikelet per spike (SPS) was significantly correlated with KNPS (r = 0.72) when averaged across all environments, the strongest correlation was observed in BHM18 (r = 0.81) (Appendix IV). Grain weight per spike (GWPS) was positively correlated with KNPS (r = 0.79), and the correlations were significant in all environments (Appendix IV). These findings indicate that SL, SPS and GWPS have a strong stable genetic association with KNPS. Even though when averaged across four test environments, TKW was significantly correlated with KNPS, the correlations were significant in two of the four environments (BHM18 and HAR18) (Appendix IV). KNPS was negatively correlated with grain length (GL) (r = -0.19) when averaged across all environments, but there was weak correlation in two environments and negative correlations in the other two

environments (Appendix IV). There was no correlation between GW and KNPS (r = 0.09) when averaged across all environments.

Table 4.2 Phenotypic correlation coefficients among yield-related traits based on trait values averaged across four environments in 2017/18 and 2018/19 seasons

Traits[a]	KNPS	SL	SPS	GWPS	TKW	GL
SL	**0.53*** **	1				
SPS	**0.72*** **	**0.73*** **	1			
GWPS	**0.79*** **	**0.54*** **	**0.62*** **	1		
TKW	0.15**	0.22*	-0.17	**0.70*** **	1	
GL	-0.19	0.13^{ns}	-0.09	0.13^{ns}	**0.39** **	1
GW	0.09^{ns}	0.22*	0.15^{ns}	**0.45*** **	**0.51*** **	**0.35** **

[a] Traits were defined in Table 4.1

Values in red, green and black, are strong, moderate and weak/no correlations among yield-related traits, respectively

*, ** and *** denotes the significance levels at $P < 0.05$, 0.01 and 0.001, respectively; ns denotes non significance at $P < 0.05$

SL was significantly correlated with SPS when averaged across test environments (r = 0.73). Although when averaged across environments, GWPS was significantly correlated with SL (r = 0.54), however the correlation was not significant in one of the four environments (ARL18) (Appendix IV). There was a weak correlation between SL and TKW (r =0.22) and SL was not significantly correlated with GL (r = 0.13). There was a weak correlation between SL and GW (r = 0.22) when averaged across four test environments. However, the correlations were significant in one of the four environments for TKW and GW, and in two of the four environments for GL (HAR18 and BHM19) (Appendix IV).

SPS was significantly correlated with GWPS (r = 0.62) and the highest correlation was in ARL18 (r = 0.62). Even though when averaged across environments, SPS was negatively correlated with TKW (r = -0.17) and GL (r = -0.09), and there was no correlation with GW (r = 0.15). However, the correlations between SPS and TKW, GL and GW were significant in one (ARL18) of the four environments (Appendix IV).

GWPS was a significantly correlated with all yield-related traits except GL (r = 0.13) when averaged across all environments, however there was weak to moderate correlations between GWPS and GL in all four environments (Appendix IV). TKW was positively correlated with GL (r = 0.39) and GW (r = 0.51) when averaged across all environments. GL was significantly correlated with GW (r = 0.35) when averaged across four environments. GL was weakly or not correlated with KNPS, SL, SPS, and GWPS, however, GL was significantly correlated with GWPS and TKW in all test environments (Appendix IV).

4.1.2 Discussion

Phenotypic variation and correlations of yield-related traits

Parental cultivars showed considerable differences for all yield-related traits in all the environments. The mean values were higher in BHM (both seasons) for all traits, except for GL. The alleles inherited from both parent cultivars contributed to the increase of yield-related traits. The large genetic variation detected in this study is due to both genetic and environmental effects and suggest that there is a possibility that QTL mapping would reveal QTLs for the studied traits. The presence of transgressive segregation also indicates the existence of genetic recombination, which indicates nicking effects of desirable alleles among the parents. Yield components are heritable traits and the estimates of heritability are higher for the SPS (67%) and lower for GW (45%), indicating that GW was significantly influenced by both genetic and environmental factors.

From our results, when traits were averaged across four environments, strong desirable correlation coefficients were detected between KNPS and SPS, KNPS and GWPS, SL and SPS and GWPS and TKW, indicate strong genetic associations between these traits. These results corresponded with the observations of Cui *et al*. (2012) who reported that SL, SPS, KNPS, and TKW have strong genetic associations with GWPS in wheat. The association between KNPS and SPS was evidently significant and positive given that, when the florets are fertile, more spikelets normally produce more grains for a single spike. Similarly, Cui *et al*. (2013) reported strong correlation coefficient between KNPS and GWPS ranging from 0.54 to 0.81 and between GWPS and TKW ranging from 0.44 to 0.71 in different environments for different populations studied. These findings indicate that KNPS and TKW may contribute more to GWPS than other yield-related traits. A positive correlation between SPS and SL agreed with other studies (Zhai *et al.,* 2016; Ma

et al., 2019) suggesting the possibility that a single allele will concurrently increase SPS and SL. The significant positive correlation implies that SPS and SL may be improved simultaneously. A negative correlation between SPS and TKW agrees with findings that an increase in SPS may decrease grain weight because of the physiological reason that a single grain will absorb fewer minerals with increased SPS that is associated with the increased KNPS due to nutrition competition (Ma *et al*., 2019). This occurrence is known as a "yield dilution" effect, where there is a negative influence of the environmental factors on mineral assimilation. In addition, traits that exhibited high and positive correlation indicate that they can be used for selection in breeding programs.

GL was strongly correlated with GW. Although previous studies reported weak correlation between GL and TKW ranging from r = 0.28 to r = 0.40 in different environments for different populations studied (Cui *et al*., 2014), our results are in agreement with (Breseghello and Sorrells, 2007), who reported strong correlations between GL and kernel weight. There was significant correlation between GW and TKW and between GW and GWPS. The results in this study agree with those of Rasheed *et al*. (2014), who reported grain width having comparatively more positive correlation with grain weight (r = 0.64) than with grain length. Similarly, Cui *et al*. (2014), reported significant positive correlation coefficients between TKW and GW, ranging from r = 0.41 to r = 0.64 in all different environments and populations studied. These results indicate that GW has more influence than GL in determining the grain weight, suggesting that grain width and 1000-kernel weight have at least some gene(s) in common.

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4.2 Experiment II: Determination of grain micronutrient (Fe and Zn) content and variation in doubled haploid lines

4.2.1 Results

Genetic variation of grain iron and zinc in South African bread wheat

Biofortification breeding efforts for Zn and Fe in bread wheat have since been steered by HarvestPlus challenge program and, have so far, resulted in the release of various cultivars in target countries. The released cultivars have improved grain yields and Zn concentrations in comparison to the conventional cultivars grown in those areas (Velu et al., 2014). To breed SA bread wheat for higher Fe and Zn, the knowledge of the existing

baseline micronutrient levels and the degree of genetic variation available within the primary genepool is fundamental.

The frequency distribution of micronutrient traits over environments showed approximately normal distributions (Figure 4.2). Iron and Zn in the DH lines varied significantly across environments (Table 4.3). The interaction of parents x environment was only significant for Zn (Table 4.3). Fe concentrations varied from 35.8 mg kg^{-1} to 112.7 mg kg^{-1}. In the same way, Zn concentrations varied from 34.3 mg kg^{-1} to 85.7 mg kg^{-1}. A significant variation was because of the environmental influences.

Values of mean, ranges and standard deviations for all traits are summarised in Appendix III, as well as mean values for parental lines. The mean values for Tugela and Elands were higher in BHM19 for Fe concentration, and Elands exhibited higher mean values compared to Tugela-DN in two environments for Fe concentration and in three environments for Zn concentration. Overall, Elands exhibited higher mean values for both mineral traits (Appendix III). The mean performance of DHs was higher in HAR18 and ARL18 for both Fe and Zn, respectively. A wide variation was observed, for instance, Fe showed a mean value of 63.2 and standard deviation of 14.5 with a minimum of 35.8 and a maximum 112.7 for samples averaged across the four environments (Appendix III). The parental cultivars had a similar trend in all four environments.

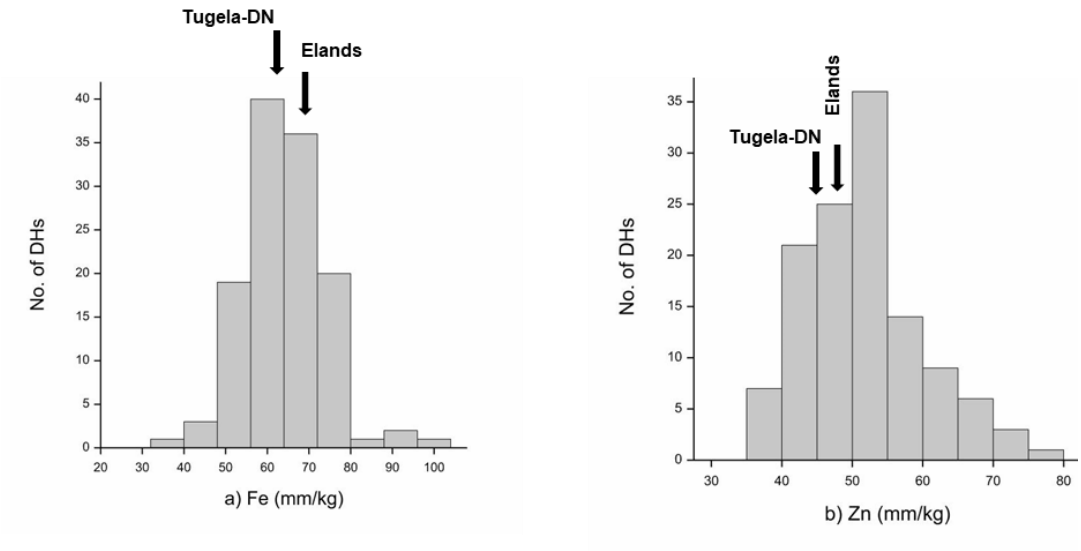

Figure 4.2 Frequency distribution of 139 DHs for Fe and Zn in two locations

Analysis of variance

Significant variability in mineral concentrations among 139 DH lines and two parental cultivars (genotypes) were found. The statistical analysis revealed significant variation for Zn concentration ($P < 0.001$) among genotypes and not significant variation for Fe concentration. The broad-sense heritability estimates for Fe was 29% and 49% for Zn (Table 4.3).

Table 4.3 ANOVA of genotype and environment effects and proportion of phenotypic variation of mineral concentrations obtained across environments among DH lines

	Mean square				Heritability
Trait[a]	Genotype	Environment	G*E	Error[b]	
Fe	256.2**	9170.4***	183.1***	53.43	0.29
Zn	307.0**	23803.7***	106.8***	22.90	0.49

[a] Traits were defined in Table 4.1

*, ** and *** denotes significance at $P < 0.05$, 0.01 and 0.001 probability levels, respectively; ns denotes non significance at $P < 0.05$

[b] Error mean squares were estimated from the check genotypes that were replicated within environments, as described in an augmented design by Federer (1961)

Correlation

Correlations among micronutrient traits measured are summarised in Table 4.4. Iron and Zn were significantly correlated ($r = 0.67$) with each other. The grain size and grain weight were anticipated to have an effect on concentrations of Zn and Fe in the grains, therefore a correlation analysis was conducted to determine their association. Grain Fe was not significantly correlated with GL ($r = 0.02$) and TKW ($r = 0.10$), and was also negatively correlated with GW ($r = -0.04$) and GWPS ($r = -0.01$) (Table 4.4). Grain Zn had a similar trend and it was not significantly correlated with GL ($r = 0.06$) and was negatively correlated with GW ($r = -0.26$), GWPS ($r = -0.29$) and TKW ($r = -0.09$). However, there were weak correlations between Fe and KNPS, SL, SPS, GWPS and TKW in BHM18 (Appendix IV).

Table 4.4 Phenotypic correlation coefficients between micronutrients and some yield-related traits based on trait values averaged across four environments in 2017/18 and 2018/19 season

Traits[a]	Fe	Zn	GL	GW	GWPS
Zn	**0.67*** **				
GL	0.02^{ns}	0.06^{ns}			
GW	-0.04	-0.26	**0.36****		
GWPS	-0.01	-0.29	0.13^{ns}	**0.45*** **	
TKW	0.10^{ns}	-0.09	**0.39****	**0.51*** **	**0.67*** **

[a] Traits were defined in Table 4.1

Values in red, green and black are strong, moderate and weak/no correlations among yield-related traits, respectively

*, ** and *** denotes significance at $P < 0.05$, 0.01 and 0.001 probability levels, respectively; ns denotes non significance at $P < 0.05$

4.2.2 Discussion

Phenotypic variation and correlations of micronutrients with yield-related traits

The availability of genetic variation of desired traits among various genotypes is a pre-requisite for a breeding programme. This permits breeders to utilise additive gene effects, transgressive segregation, and heterosis to improve micronutrient density (Pfeiffer and McClafferty, 2007). However, the bread wheat germplasm has a narrow range for grain Fe and Zn concentrations. Badakhshan *et al*. (2013) reported a grain Fe ranging from 41.4 mg/kg to 67.7 mg/kg, and grain Zn ranging from 36.4 mg/kg to 73.8 mg/kg in a study among 81 cultivars of bread wheat. Similarly, Srinivasa *et al*. (2014) reported Fe and Zn concentration ranges from 25.3 mg/kg to 59.5 mg/kg and 18.8 mg/kg to 73.5 mg/kg, respectively, across RILs population. Gorafi *et al.* (2016) reported Fe concentrations ranging from 26.6 mg/kg to 57.6 mg/kg in the first season and 22.2 mg/kg to 78.5 mg/kg in the second season; and Zn concentrations ranging from 27.0 mg/kg to 65.8 mg/kg in the first season and 20.6 mg/kg to 65.8 mg/kg in the second season. Although the grain Fe and Zn concentrations in Tugela-DN and Elands were in the same range as reported for the cultivated hexaploid wheat germplasm (Badakshan *et al.,* 2013), the grain Fe and Zn concentrations in Elands were relatively higher than in Tugela-DN. This can be explained from the fact that Tugela-DN has high yielding potential than Elands and it is common

knowledge that yield and nutrition are negatively correlated, as it is evident in our results. This large genetic variation detected in our study for DHs, suggests that there is a possibility that QTL mapping would reveal QTLs for the studied traits. Most of the DH lines outperformed the parents for all traits, suggesting transgressive segregation and indicating polygenic inheritance as expected for quantitative traits. The presence of transgressive segregation indicates the existence of diverse sets of genes in the parental lines for the target traits. Additionally, the results indicate the occurrence of large variation among the DHs under the study.

Micronutrients are heritable traits and the estimates of heritability are higher for the Zn (49%) and lower for Fe (29%), indicating that these minerals were significantly influenced by both genetic and environmental factors. The results from our study suggest that there is considerable variability for Zn concentrations for selection among genotypes evaluated (Appendix IV). The results suggest that there is variation in the ability of each genotype to assimilate minerals from the soil through roots, translocate and redistribute them to wheat grains. In addition to variation being attributed by genetic ability of genotypes, soil properties such as water content, soil pH, organic matter, redox conditions, etc., also play an important role in controlling how much of these micronutrients are available for accumulation by the crops (Shuman, 1998; Frossard *et al.,* 2000; Sperotto *et al.,* 2014).

From our results, when traits were averaged across four environments, significant desirable correlation coefficients were observed between Fe and Zn. In agreement with our results, Liu *et al.* (2019) observed strong positive correlation (r = 0.517) between grain Zn and grain Fe concentrations. Other previous studies have reported that grain Fe and Zn have positive associations in wheat (Peleg *et al*., 2009; Velu *et al*., 2011; Gorafi *et al*., 2016; Velu *et al*., 2019). These results suggest that the alleles for Zn and Fe accumulation in wheat grains co-segregate or have pleiotropic effect, and therefore these minerals can be enhanced simultaneously.

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4.3 Experiment III: Bi-parental QTL map construction using SNP markers on DH population

4.3.1 Results

SNP marker distribution in the linkage map

As mentioned in section 3.3.3.2, a total of 3 204 markers were genotyped of which 1 742 markers were monomorphic in this DH population, signifying the two parents sharing most of the marker alleles. These markers were removed from the analysis. Therefore, the preliminary genetic linkage map for DH population consisted of 1 462 SNP marker loci. After a filtering process of all redundant and non-informative markers on IciMapping, only 318 SNP markers were utilised for the creation of a final genetic linkage map. All 318 SNP markers were linked to the 21 wheat chromosomes, which were represented by the linkage groups (Figure 4.3). The SNP markers covered 11 538.21 cM of the wheat genome (Figure 4.4). The D genome had most of the SNPs assigned to it, with 148 (4 390.92 cM) SNPs in total, while the A and B genomes harboured 81 (3 784.25 cM) and 89 (3 366.04 cM) SNPs (Table 4.5). A schematic representation of effect-plots, to illustrate how QTLs contributed by alleles inherited from parents were determined, was shown in Figure 4.5.

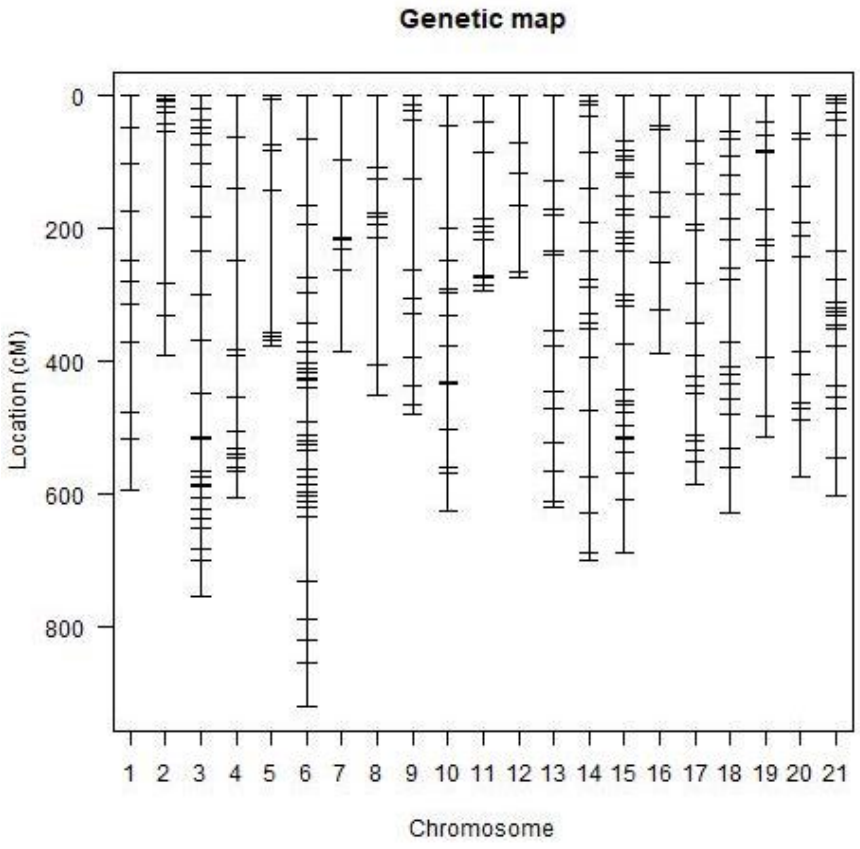

Figure 4.3 Genetic map representing 21 chromosomes of wheat

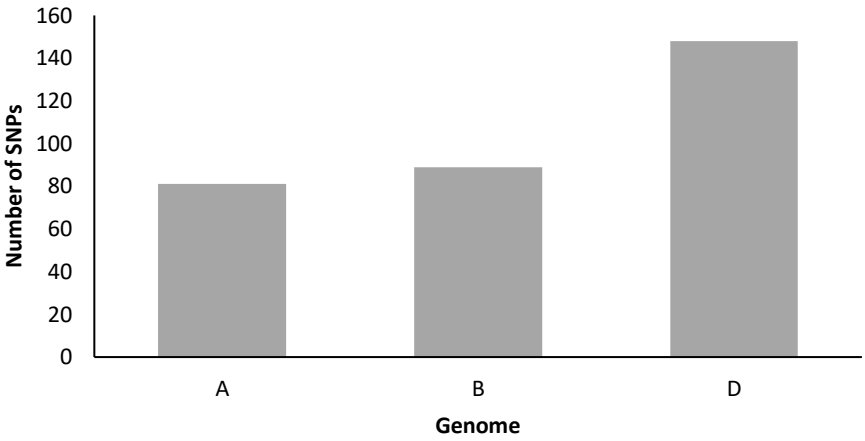

Figure 4.4 Number of SNP GBS-based markers assigned on A, B and D genome of wheat

Mapping of zinc (Zn)

A total of six putative QTLs associated with Zn concentration were identified and were located on chromosome 2D (two QTL), 5B, 5D, 6A and 6B, respectively. Among them one major QTL, *QZn.sg-6A.2,* which was found between marker interval *cUAm0425-c6Am0024*, and was consistent in two different environments (Table 4.6; Figure 4.6), with alleles contributed from Elands (Table 4.7). This QTL explained 16.0% of the phenotypic variance. Five putative minor QTLs were detected and were designated as *QZn.sg-2D.1a, QZn.sg-2D.1b, QZn.sg-5B.1, QZn.sg-5D.1* and *QZn.sg-6B.1* were identified with LOD score ranging from 2.02 to 2.82, explaining between 7.2% and 19.3% of the phenotypic variation (Table 4.6). Both QTL identified on chromosome 2D were identified in one environment (ARL18) and were in close proximities.

Mapping of iron (Fe)

A total of five QTLs associated with Fe concentration were identified on chromosome 2D (two QTLs), 5B, 5D and 7D with additive genetic effects involved in iron (Fe) concentrations when evaluated across four different environments at a threshold of LOD > 2.0 (Appendix V). Among them, one QTL designated as *QFe.sg-7D.2* was consistent in two different environments, this QTL was found between marker interval *c7Dm0047-c7Dm0041*. The QTL had an LOD score of 2.21, explaining 20.0% of the phenotypic variation (Table 4.6) and with alleles inherited from Tugela-DN (Table 4.7). Four putative minor QTLs, designated as *QFe.sg-2D.1a, QFe.sg-2D.1b, QFe.sg-5B.1, QFe.sg-5D.1,*

were identified with LOD scores of 2.91, 2.91, 1.84 and 1.92, explaining 20.9%, 21.0%, 4.4% and 1.5% of the phenotypic variation, respectively (Table 4.6; Appendix V). Both QTLs identified on chromosome 2D were identified in one environment (ARL18) and were in close proximities.

Table 4.5 SNP GBS-based markers distributed on different chromosomes in the genetic linkage map

Linkage group	Chromosome	Length (cM)	No. of SNPs
1	1A	592.06	11
2	1B	389.79	10
3	1D	752.12	27
4	2A	603.85	14
5	2B	375.03	9
6	2D	919.73	33
7	3A	383.57	7
8	3B	451.62	9
9	3D	528.87	12
10	4A	685.79	14
11	4B	292.95	11
12	4D	274.54	6
13	5A	617.71	14
14	5B	699.07	20
15	5D	686.99	31
16	6A	387.1	9
17	6B	583.35	17
18	6D	627.18	19
19	7A	514.17	12
20	7B	574.23	13
21	7D	601.49	20
Total	21	11 541.21	318

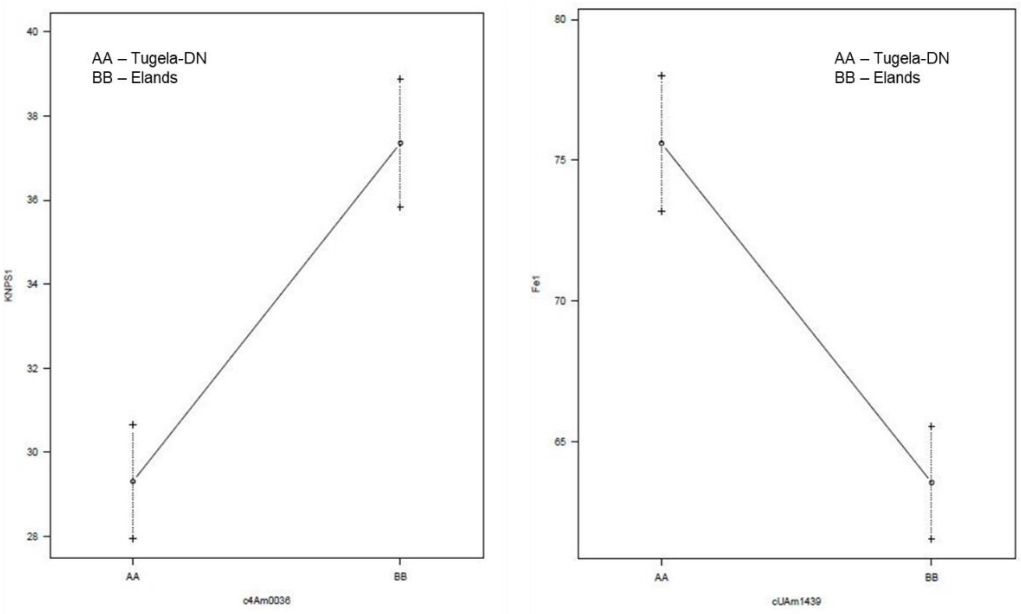

Figure 4.5 Example of effect-plots used to determine the parent where alleles were inherited.

Mapping of grain weight per spike (GWPS)

Fifteen QTLs were detected on chromosomes1A (two QTLs), 2B, 2D (two QTLs), 4A, 5B (two QTLs), 5D (four QTLs), 6A (two QTLs) and 6B with additive genetic effects involved in grain weight per spike (GWPS) when evaluated across four different environments at a threshold of LOD > 2.0 (Appendix V). Among them, two major QTLs, designated as *QGwps.sg-5D.2d* and *QGwps.sg-6B.2,* with LOD scores of 2.56 and 2.52, each explaining the phenotypic variation of 7.5%, and 5.0%, respectively (Table 4.6; Figure 4.6; Appendix V). These QTLs were found consistent in two different environments and the alleles were contributed by Tugela-DN (Table 4.7). Thirteen putative minor QTLs were identified with LOD scores ranging from 1.94 to 2.49, explaining the phenotypic variation ranging between 2.0% and 17.8% (Table 4.6; Appendix V). The minor QTLs were found to be inconsistent and varied with the test environments.

Table 4.6 Summary of QTLs identified and co-located for micronutrient and yield-related traits using 139 DHs across environments during two seasons (2017/18 and 2018/19)

Chr	Marker Peak / Interval	Pos (cM)	Env	GWPS			SL			SPS			KNPS			TKW			GL			GW			Fe			Zn		
				LOD[a]	PVE (%)[b]	Add[c]	LOD[a]	PVE (%)[b]	Add[c]	LOD[a]	PVE (%)[b]	Add[c]	LOD[a]	PVE (%)[b]	Add[c]	LOD[a]	PVE (%)[b]	Add[c]	LOD[a]	PVE (%)[b]	Add[c]	LOD[a]	PVE (%)[b]	Add[c]	LOD[a]	PVE (%)[b]	Add[c]	LOD[a]	PVE (%)[b]	Add[c]
1A	c1Am0043-c1Am0052	274.0	BHM18	2.07	2.7	-0.22										2.05	11.7	-3.10												
	c1Am0052	278.0	BHM18	2.19	3.5	-0.13										2.03	10.1	-2.89												
2D	cUAm1439-c2Dm0042	316.0	ARL18 BHM18 HAR18	2.26	2.0	-0.09										2.06	14.6	-2.56				1.84	13.0	-0.14	2.91	21.0	-6.61	2.65	19.3	-5.88
3A	c3Am0013-c3Am0070	42.0	BHM19 BHM18 ARL18																2.62 1.75 1.69	9.1 8.0 8.9	0.40 0.37 0.25	2.16	5.9	0.11						
4A	c4Am0036	60.7	ARL18	2.49	10.7	0.11				2.48	13.4	0.64	3.84	13.4	3.36															
5B	cUAm1428-c5Bm0069	72.0	ARL18 BHM18	2.19	15.4	0.11				2.24 2.04	4.8 0.8	0.93 -0.45	 1.76	 2.8	 -1.46							2.02	11.9	0.22	1.84	4.4	-7.51	2.31	14.5	-5.27
5D	c5Dm0043-cUnkm0040	74.0	ARL18	2.29	16.1	0.12				1.89	15.8	0.66	2.22	15.7	3.56															
	cUnkm0040-cUAm1314	86.0	ARL18	2.15	15.2	0.12							2.33	16.4	3.47										1.92	1.5	-5.22	2.02	15.1	-5.23
	cUAm1314-cUAm1178	94.0	BHM18 BHM18 BHM19	1.94 2.56 1.17	17.8 7.5 6.6	-0.19 -0.24 -0.07				2.47 2.02	3.5 1.1	-0.47 -1.05	3.21 3.73 1.83	17.7 20.2 8.4	-0.18 -4.29 -1.91															
6A	cUAm0425-c6Am0024	92.0	BHM18 HAR18																			2.02	4.8	-0.18				1.71	16.0	7.39
	c6Am0013-c6Am0012	206.0	BHM18 BHM18	2.43 2.37	7.1 7.5	-0.32 -0.33										2.45 2.53	13.8 14.2	-4.52 -1.56												
6B	c6Bm0015-c6Bm0051	12.0	BHM18							1.80	6.6	0.83	1.86	13.3	3.66													2.82	7.2	-6.19
6D	c6Dm0021-c6Dm0006	476.0	BHM18 HAR18																			1.89	5.7	-0.09						
7A	c7Am0065-cUAm0764	190.0	HAR18				2.55	11.4	0.88	2.59	8.7	1.20																		
7D	c7Dm0047-c7Dm0041	44.0	BHM18 HAR18																						2.21	20.2	-13.0			

Chr – Chromosome, Pos – Position, cM – Centi Morgan, Env – Environment;

[a]LOD – Logarithm of odds, where values are the peak logarithm of odds score for the given QTL;

[b]PVE – Phenotypic variance explained, where values indicate the phenotypic variation explained by the QTL;

[c]ADD – Additive, where values indicate the additive effect of the QTL; positive and negative effects indicate that the QTL alleles were contributed by Elands and Tugela-DN, respectively.

Mapping of spike length (SL)

Nine QTLs were identified on chromosome 2D, 3D, 4A, 4B, 5D, 6A, 7A and 7B (two QTLs) with additive genetic effects involved in spike length (SL) when evaluated across four different environments at a threshold of LOD > 2.0 (Appendix V). Among them, one major QTL designated as *QSl.sg-7B.2,* was detected in two different environments with LOD score of 1.59, explaining 11.1% of the phenotypic variation (Table 4.6). Seven putative minor QTLs were identified with LOD scores ranging between 2.12 and 2.78, explaining between 1.4% and 16.5% of the phenotypic variation (Table 4.6; Appendix V). These putative minor QTLs were found to be inconsistent and varied with the test environments.

Mapping of spikelet per spike (SPS)

Twelve QTLs were discovered on chromosomes 3A, 4A, 5B (two QTLs), 5D (four QTLs), 6B (two QTLs), 7A and 7B with additive genetic effects involved in spikelet per spike (SPS) when evaluated across four different environments at a threshold of LOD > 2.0 (Appendix V). Among them, one major QTL designated as *QSps.sg-5B.2a,* was found between marker interval *cUAm1428-c5Bm0069* and was detected in two different environments with LOD score of 2.24, explaining 4.8% of the phenotypic variation (Table 4.6). Eleven putative minor QTLs were detected with LOD scores ranging between 1.63 and 2.59, explaining between 1.1% and 16.1% of the phenotypic variations (Appendix V).

Mapping of kernel number per spike (KNPS)

Ten QTLs were detected on chromosomes 1A, 2B, 4A, 5B (two QTLs), 5D (four QTLs) and 6B with additive genetic effects involved in kernel number per spike (KNPS) when evaluated across four different environments at a threshold of LOD > 2.0 (Appendix V). Three major QTLs were detected. The first major QTL, designated as *QKnps.sg-4A.1,* was linked to a marker *c4Am0036* and was detected in ARL18 with LOD score of 3.81 explaining 19.5% of the phenotypic variation (Table 4.6; Figure 4.6; Appendix V). The alleles for this QTL were inherited from Elands (Table 4.7). The second QTL designated as *QKnps.sg-5D1c* was found between marker interval *cUAm1314-cUAm1178* and was detected in two different environments with LOD scores of 3.21, explaining 17.7% of the phenotypic variations, respectively (Table 4.6; Figure 4.6; Appendix V). Additionally, Elands

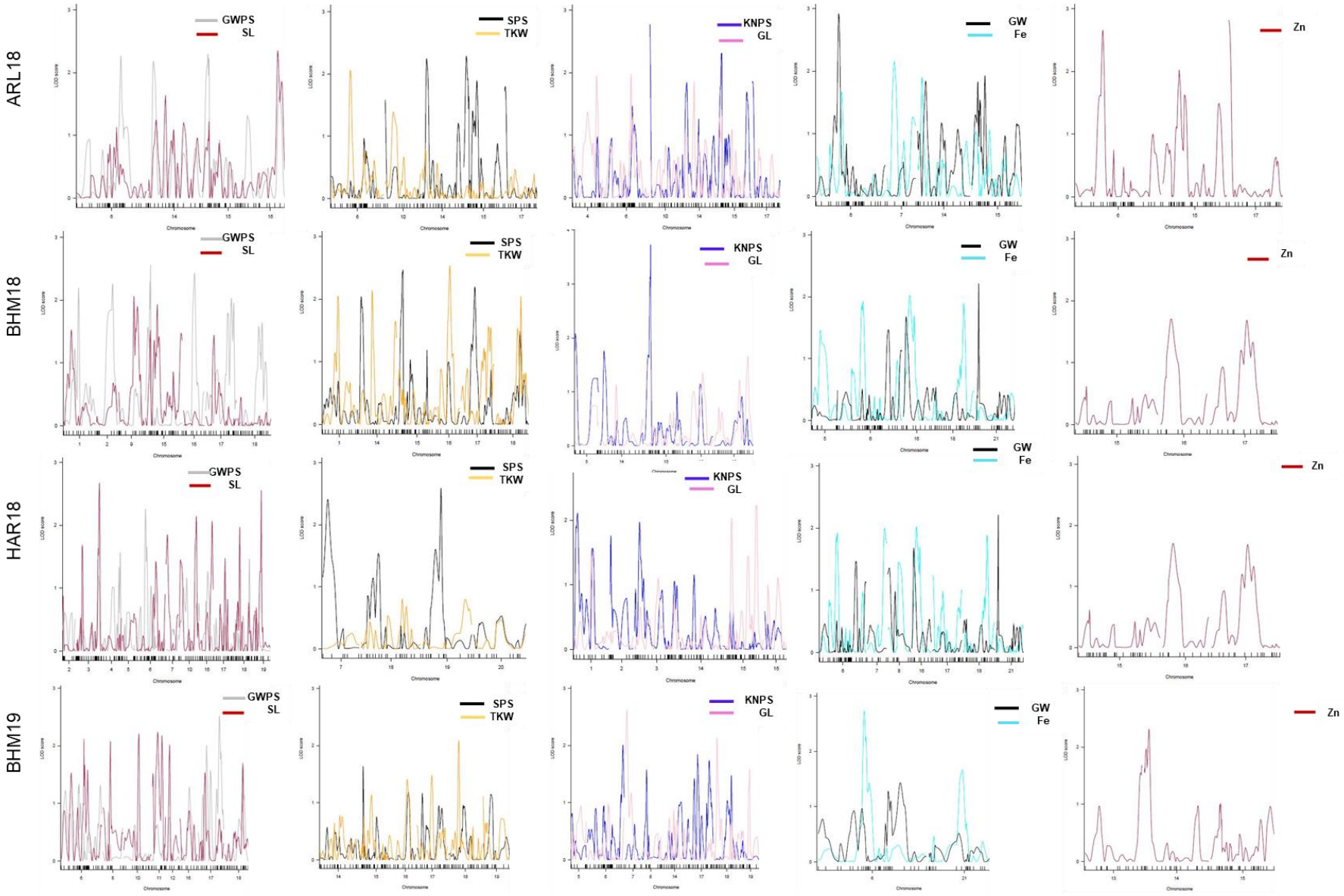

Figure 4.6 Schematic representation of QTL LOD peaks on different chromosomes for all traits evaluated in 2017/18 and 2018/19 seasons

contributed alleles for this QTL. The last major QTL designated as *QKnps.sg-5D.2d* was found between marker interval *cUAm1314-cUAm1178* and was detected in two different environments with LOD scores of 3.73 and 1.83, explaining 20.2% and 8.4% of the phenotypic variations, respectively (Table 4.6; Figure 4.6; Appendix V), and the alleles for this QTL were inherited from Tugela-DN (Table 4.7). Seven putative minor QTLs were detected with LOD scores ranging between 1.76 and 2.33, explaining between 1.3% and 16.4% of the phenotypic variations (Table 4.6).

Table 4.7 A list of actual SNP alleles for the significant QTL detected through QTL mapping and the parent where the allele was inherited

QTL	Marker Interval/Peak	SNP alleles	Parent
QGwps/Tkw.sg-1A.1a	c1Am0043-c1Am0052	C>G - C>T	Tugela-DN
QGwps/Tkw.sg-1A.1b	c1Am0052	C>T	Tugela-DN
QGwps/Tkw/Gw/Fe/Zn.sg-2D.1b	cUAm1439-c2Dm0042	G>A - T>C	Tugela-DN
QGl/Gw.sg-3A.3	c3Am0013-c3Am0070	G>C - A>G	Elands
QGwps/Sps/Knps.sg-4A.1	c4Am0036	A>G	Elands
QGwps/Sps/Knps/Gw.sg-5B.1a	cUAm1428-c5Bm0069	G>A - G>A	Tugela-DN and Elands
QGwpsSpsKnps.sg-5D.1a	c5Dm0043-cUnkm0040	A>G - G>C	Elands
QGwps/Knps/Fe/Zn.sg-5D.1b	cUnkm0040-cUAm1314	G>C - C>T	Elands
QGwps/Sps/Knps.sg-5D.1d	cUAm1314-cUAm1178	C>T - G>A	Tugela-DN
QGw/Zn.sg-6A	cUAm0425-c6Am0024	T>C - C>T	Tugela-DN and Elands
QGwps/Tkw.sg-6A.1a	c6Am0013-c6Am0012	A>C - A>C	Tugela-DN
QSps/Knps/Zn.sg-6B.1a	c6Bm0015-c6Bm0051	C>G - C>T	Tugela-DN and Elands
QGw.sg-6D.2a	c6Dm0021-c6Dm0006	T>C - T>A	Tugela-DN
QSl/Sps.sg-7A.1	c7Am0065-cUAm0764	G>A - T>C	Elands
QFe.sg-7D	c7Dm0047-c7Dm0041	C>T - C>T	Tugela-DN

Mapping of 1000-kernel weight (TKW)

Ten QTLs were mapped on chromosome 1A (two QTLs), 2A, 2D, 4B, 5B, 6A (two QTLs) and 6D (two QTLs) with additive genetic effects involved in 1000-Kernel weight (TKW) when evaluated across four different environments at a threshold of LOD > 2.0 (Appendix V). All QTLs were minor putative QTLs and were detected with LOD scores

ranging between 1.69 and 2.53, explaining between 5.2% and 14.6% of the phenotypic variation (Appendix V). All the QTLs were found common in one environment only.

Mapping of seed size

For grain length, five QTLs were mapped on chromosome 2A, 2D, 3A, 5B and 5D with additive genetic effects involved in grain length (GL) when evaluated across four different environments at a threshold of LOD > 2.0 (Appendix V). Two major QTLs were detected. The first major QTL designated as *QGl.sg.3A.3,* was found between marker interval *c3Am0013-c3Am0070* and was detected in three different environments with LOD scores of 2.62, 1.75 and 1.69, and explaining 9.13, 7.98 and 8.91% of the phenotypic variations, respectively (Table 4.6; Figure 4.6 Appendix V). The alleles for this QTL were contributed by Elands (Table 4.7). Another major QTL was found between marker interval *c5Dm0048-c5Dm0007* and was detected with LOD score of 2.23 and explaining 20.7% of the phenotypic variation (Table 4.6). Three minor putative QTLs were detected with LOD scores ranging between 1.86 and 1.98, explaining between 2.1% and 13.9% of the phenotypic variation, respectively (Table 4.6; Appendix V).

For grain width, eight QTLs identified on chromosome 2D (two QTLs), 3A, 5B (two QTLs), 5D, 6A and 6D with additive genetic effects involved in grain weight (GW) when evaluated across four different environments at a threshold of LOD > 2.0 (Appendix V). Two major QTLs were detected. The first major QTL designated as *QGw.sg.3A.1,* was found between marker interval *c3Am0013-c3Am0070* with LOD score of 2.27, explaining 14.4% of the phenotypic variations (Table 4.6, Figure 4.6; Appendix V). Another QTL designated as *QGw.sg.6D.1,* was found between marker interval *c6Dm0021-c6Dm0006* and was detected in two different environments with LOD score of 1.89 and explaining 5.7% of the phenotypic variation (Table 4.6 Figure 4.6; Appendix V). Six putative minor QTLs were identified with LOD scores ranging from 1.84 to 2.73 and explaining 3.6 to 13.0% of the phenotypic variation (Table 4.6; Appendix V).

4.3.2 Discussion

A genetic linkage map of DH population

The GBS is a preferred high-throughput genotyping method due to its ability to detect and identify large numbers of markers. The technique has the ability to evaluate large sets of known markers that can be used in genetic analysis for breeding purposes (Poland and Rife, 2012; Peterson *et al*., 2014). This method has been utilised to develop a high-density genetic map. In the present study, a Tugela-DN/Elands DH population of 139 genotypes was used to construct a genetic map. A genetic map was successfully created using SNP markers from a GBS platform. The GBS has been applied to construct saturated maps in wheat (Wang *et al*., 2014), rice (Huang *et al*., 2009), soybean (Jarquin *et al*., 2014) and maize (Zhang *et al*., 2015a).

All 21 wheat chromosomes were represented evenly by the linkage groups, with markers assigned throughout the three genomes (A, B and D). The D genome contained more loci (Figure 4.5). This finding differs from most hexaploid wheat maps in which fewer markers were found in the D genome but similar to the results of Cui *et al*. (2014). However, chromosome 4D contained fewer markers, which concurs with other hexaploid wheat maps (Akbari *et al*., 2006). Most markers were mapped on the A genome (37.5%) and the D genome (35%), the remaining markers (27.5%) were mapped on the B genome.

QTL analysis of yield-related traits in DH bread wheat genotypes

Grain yield is one of the most complex traits with the environmental effects having strong influences. Moreover, the low heritability of grain yield has delayed the advancement in our understanding of genes and gene pathways that regulate this trait. Therefore, a strategy to tackle this challenge is to study this trait by focusing on its related traits (Kuzay *et al*., 2019). There are requirements for a QTL to be beneficial for MAS. A QTL is supposed to be realised in most tested environments and in the germplasm where genotypes segregating for the traits. None of the previously mapped QTLs were discovered in this study. All the putative QTLs detected in the present study responsible for yield component traits had not been reported before in previous studies, but some were detected at close proximities to the locations of known genes/QTLs. Therefore, it is speculated that the Tugela-DN/Elands population potentially harbours unexploited genes of yield component traits in wheat. According to field evaluations of four environments in 2018 and 2019, the major QTLs detected were in the 2D, 3A, 5B and 5D chromosomes.

Grain weight per spike, 1000-kernel weight, and grain size

Fifteen QTLs were detected for GWPS, but of interest may be the QTL *QGw*ps*.sg-4A.1,* which shared this interval with *QSps.sg-4A.1* as well as *QKnps.sg-4A.1,* showing a pleiotropic QTL or a gene-rich region. This was expected because these traits were found correlating significantly. The QTL on chromosome 4A explained a total of 37.5% of the phenotypic variance, was linked to SNP marker, *c4Am0036,* and the QTL was contributed by alleles inherited from Elands (Table 4.7). However, the QTL was not stable because it was found in one test environment. These findings conflict with Börner *et al*. (2002) and Heidari *et al*. (2011) who reported this QTL and both studies found it stable across different environments; therefore, the QTL is worth further exploration. QTL designated as *QGw*ps*.sg-5B.1* co-localised with *QGw.sg-5B.1*, it was expected because there was a significant and positive correlation between these traits. Additionally, a major QTL *QGw*ps*.sg-5D.2d* as well as *QKnps.sg-5D.2* shared this interval, indicating a pleiotropic QTL or a gene-rich region. These two traits (GWPS and KNPS) were also found to be significantly and positively correlating, indicating that they can be selected together in the breeding programme. Previous studies found QTLs on chromosomes 1A (Cui *et al*., 2013; Heidari *et al*., 2011), 2D (Heidari *et al*., 2011), 5B (Cui *et al*., 2013; Heidari *et al*., 2011), 5D (Cui *et al*., 2013), which corroborate the involvement of these chromosomes found in our study. Similar to our study, Patil *et al*. (2013) reported a QTL on chromosome 6A that was common in one location. Ten of the QTLs for GWPS were contributed by alleles inherited from Tugela-DN, with the remaining five QTLs contributed by alleles inherited from Elands, indicating that Tugela-DN potentially harbours genes that can further be explored in MAS breeding.

Ten QTLs were identified for TKW. The QTL, *QTkw.sg-6D.1a,* co-localised with QTL for GW, *QGw.sg-6D.1a*, showing that breeding for one trait on this genomic region would not impede progress on the other trait. However, this QTL was detected in one environment, therefore it was not stable. Previous studies found QTLs on chromosome 2A (Tsilo *et al*., 2010; Krishnappa *et al*., 2017; Wei *et al*., 2014), 5B (Tsilo *et al*., 2010; Ramya *et al*., 2010; Krishnappa *et al*., 2017; Zhang *et al*., 2016b) and 7A (Tsilo *et al*., 2010) in different mapping populations, which corroborate the involvement of these chromosomes found in our study. QTLs on chromosome 2A, 5B and 7A for TKW co-localised with QTLs for kernel diameter and kernel size distribution (Tsilo *et al*., 2010). Li *et al*. (2015) reported QTLs on chromosomes 4B, 5B and 6A, this study corroborates the involvement of chromosomes 4B and 5B found in our study. QTLs on chromosome 6D, have not been reported before in previous studies.

Five QTLs were identified for GL. Of interest was a major QTL *QGl.sg-5D.1* that explained 20.69% of the phenotypic variation. Although this QTL was stable in one environment, it is worth exploring further through fine-mapping to determine genes that determine its effects. A major QTL on chromosome 3A for GL, which was stable in three environments co-located with a QTL for GW in ARL18, indicating that these traits share some gene(s) and could be improved simultaneously. This was expected because these traits were found correlating significantly.

QTL mapping detected eight putative QTLs for GW. A QTL designated as *QGw.sg-6D.1*, which was stable in two different environments and was found co-locating with *QTkw.sg-6D.1.* This was expected because these traits were found to be positively correlating, showing a pleiotropic QTL or a gene-rich region and suggesting that these traits could be increased simultaneously. QTL on chromosome 5B for GW co-located with a QTL for GWPS, indicating that these traits can be improved simultaneously. Previous studies reported QTLs for GL and GW on different chromosomes; for instance, Cui *et al*. (2016) identified QTLs on chromosome 1B, 2A and 2D. Breseghello and Sorrels, (2007) reported QTLs on 1B, 2D and 5B. Li *et al*., (2015) reported QTLs on 1B, 1D, 3D, 4B, 5B, and 6A. These studies corroborate the involvement of some of the chromosomes (2A, 2D, 5B and 6A) found in our study, although the QTLs were located on different positions. To the best of our knowledge, no QTL for grain length has been detected on chromosome 3A. Therefore, the Tugela-DN/Elands population under the study could potentially harbour the unexploited genes for grain size. This could be because both Tugela-DN and Elands cultivars have medium to high yield potential.

Spike length, Spikelet per spike, Kernel number per spike

QTL mapping detected major QTL on chromosome 7A and 7B for SL. The QTL *QSl.sg-7A.1* co-located with QTL for SPS. This is not a surprise as these two traits were found to be positively and significantly correlating in all environments. The QTL *QSl.sg-7B.1* was stable in two different locations, indicating that this QTL could potential harbour genomic region that can be explored in MAS breeding. The QTL identified on chromosome 4A suggest the importance of this QTL in governing the trait which agrees well with the previous reports by Cui *et al*. (2012), Wang *et al*. (2011), and Patil *et al*. (2013) in bread wheat. Other previous studies found QTLs on chromosome 2D (Zai *et al*., 2016; Wang *et al*., 2011; Heidari *et al*., 2011), 3D (Jaiswal *et al*., 2016), 6A (Heidari *et al*., 2011) 7B (Zai *et*

al., 2016), which corroborate the involvement of these chromosomes found in our study. Both parental cultivars in our study contributed alleles equally to the QTLs for SL.

QTL mapping detected eight QTLs for increased SPS in our study. Of interest is the QTL on chromosome 5B, which was stable in two different environments. This QTL designated *QSps.sg-5B.2a* was found in the same marker interval as QTLs detected for GWPS, KNPS, GW and Zn concentration. It was expected for yield-related traits to co-locate because they were also found to be positively correlated with each other, indicating that they have strong genetic associations. However, for *QSps.sg-5B.2a* to be found on the same marker interval as *QZn.sg-5B.1* rather calls for further investigations because these traits were found to be negatively correlating, as mentioned before. Previous studies found QTLs on chromosome 5D (Ma *et al*., 2019), 7A (Cui *et al*., 2012; Zhang *et al*., 2015b; Kuzay *et al*., 2019) and 5B (Liu *et al*., 2006; Ma *et al*., 2019). Total of 11 QTLs were detected for SPS, however ten of the QTLs were not consistent across test environments, suggesting that the environment had more influence on this trait. Furthermore, the minor putative QTLs detected in this study were found either on the same chromosome or in close proximity with those reported in previous studies. Eight of the QTLs were contributed by alleles inherited from Elands and the remaining four were contributed from Tugela-DN, indicating that Elands has potential genomic regions that may contribute towards yield improvement through MAS.

Ten QTLs were identified for increased KNPS in our study. Previous studies found QTLs on chromosome 1A (Heidari *et al*., 2011), 2B (Heidari *et al*., 2011; Gao *et al*., 2015; Shi *et al*., 2017), 4A (Gao *et al*., 2015; Cui *et al*., 2017; Shi *et al*., 2017), which corroborate the involvement of these chromosomes found in our study. Cui *et al*., 2017 found a stable QTL on chromosome 4A, which was verified in 10 different environments using three different QTL mapping softwares. A major QTL on chromosome 4A for KNPS, which was linked to a SNP marker *c4Am0036*, was found co-locating with QTLs for SL and SPS; this was no surprise as these traits were found to be significantly and positively correlating in all test environments. This further proves that these traits can be selected simultaneously for breeding purposes.

Noteworthy, most of the QTLs detected in our study were consistent between one and three locations but not in all the test environments. However, they met the statistical requirements, i.e. high LOD and PVE values (Table 4.6).

QTL analysis of micronutrient traits in DH bread wheat genotypes

Five QTLs were identified (*QFe.sg-2D.1a, QFe.sg-2D.1b, QFe.sg-5B.1, QFe.sg-5D.1* and *QFe.sg-7D.1*) for Fe. QTLs on chromosome 2D for Fe (*QFe.sg-2D.1a, QFe.sg-2D.1b*) shared these intervals with *QZn.sg-2D.1a* and *QZn.sg-2D.1b,* respectively, indicating pleiotropic QTLs or gene-rich regions. The first QTL on chromosome 2D (1a) explained 40.2% of the phenotypic variation and the second QTL (1b) explained 39.8% of the phenotypic variation. However, these QTLs were not stable because they were found in one environment. Similarly, another QTL for Fe was identified on chromosome 5D, designated as *QFe.sg-5D.1,* and co-located with *QZn.sg-5D.1*. Our results concur with those of Gorafi *et al*. (2016), who detected QTLs for both Fe and Zn on chromosomes 2D and 5D. It was expected for micronutrient traits to be detected on the same loci because they were also found to be significantly and positively correlating, indicating that the presence of the other can be used to predict the other, and they can be selected together in the breeding programme. A QTL detected on chromosome 7D explained 20.2% of the phenotypic variation. Roshanzamir *et al.* (2013) reported a QTL on chromosome 7D for Fe, which corroborates the findings of our study. All QTLs for Fe were contributed by alleles inherited from Tugela-DN.

A QTL on chromosome 5B, designated as *QFe.sg-5B.1,* was found co-locating with a QTL for GW. Another QTL on chromosome 6A, designated as *QZn.sg-6A.1,* was found co-locating with a QTL for GW. Another QTL on chromosome 6B, designated as *QZn.sg-6B.1*, was found co-locating with QTLs for KNPS and SPS in BHM18. The very same QTL on chromosome 6B was mapped by Velu *et al*. (2017) for high grain Zn using two different wheat RIL mapping populations (tetra- and hexaploid). This study corroborates the involvement of chromosomes 6B found in our study. These results suggest that there is a possibility of improving both micronutrients and other yield-related traits simultaneously. Five of the six QTLs for Zn were contributed by alleles inherited from Tugela-DN.

Very few mineral QTLs were identified on the same genomic regions as yield-related traits. For instance, both Fe and Zn QTLs on chromosome 2D were found co-locating with TKW (Table 4.6). Hao *et al*. (2014) reported a QTL on the centromere of chromosome 2B, which had a pleiotropic effect and can increase TKW at significant level.

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CHAPTER 5

CONCLUSIONS

5. Chapter outline

This is the final chapter of the thesis and it represents the concluding remarks, along with limitations and recommendations. The conclusions presented are linked to the discoveries of the study and proposes solutions to the research problem. The last part of the chapter are the suggestions and recommendations for future research and the contribution of this study to the research community.

5.1 Concluding remarks

The study showed considerable genetic variability for all traits studied and grain Fe and Zn concentrations and these traits were significantly and positively correlated in all environments. However, micronutrient concentrations were either negatively correlated or there were no significant correlations with all yield-related traits. These results validate the challenge faced when efforts are made to increase yield and nutrient concentrations simultaneously. These traits are among the most important traits in wheat targeted in breeding programmes to address the high numbers of people suffering from malnutrition and the expected increase in the world population. The inheritance of these traits is known to be complicated. Therefore, the study of the genetics of such multiple traits becomes possible performing QTL analyses. This study conducted such analyses for nutritional quality and yield-related traits and QTLs were detected in a doubled haploid bread wheat population. According to field evaluations of four environments in 2018 and 2019, the consistent QTL detected for nutritional quality and yield-related traits were detected in the 2D, 3A, 4A, 5B and 5D chromosomes. The QTL on chromosome 4A for GWPS, SPS and KNPS was linked to the SNP marker *c4Am0036*, should be explored further by means of fine-mapping of this specific regions. Additionally, the genomic regions in marker intervals for QTLs on chromosomes 2D, 3A, 5B and 5D should also be investigated by means of fine-mapping. This can lead to increases in genetic progress in breeding through strategies such as MAS. Evidence suggests that mineral concentration is diluted as yield potential increases. However, in our study a QTL for grain Zn and Fe concentrations detected in chromosome 2D were in the same marker intervals as GWPS, TKW and GW in different environments. Another QTL for grain Fe concentration in chromosome 5B was in the same marker intervals as GWPS, SPS and GW in ARL18, interestingly *QSps.sg-5B.2* was stable in two environments. The marker intervals for these genomic regions associated with stable

and most consistent QTLs may be exploited in early breeding selections to improve these traits. From this study we can conclude that the nutritional quality variation does in fact exist in South African bread wheat genotypes and the genomic regions responsible were detected using bi-parental QTL mapping. Furthermore, there were QTLs detected on several chromosomes (6D for TKW, 3A for GL, 5B for Fe, and 6A for Zn) that have not been reported in previous studies. This emphasizes the need for more research to be carried out to evaluate more South African wheat cultivars.

5.2 Limitations and recommendations

To the best of our knowledge, there has not been any studies reported on variation of nutritional quality on South African bread wheat genotypes, particularly on Fe and Zn minerals and the genomic regions influencing their genetic variation. From the results of this study SNP GBS-based markers proved to be useful in detecting stable QTLs, therefore we recommend fine-mapping of the stable QTLs using association mapping. Another recommendation is for research collaborations to be formed in South Africa and around the world in order to improve the nutritional value of this challenging wheat crop. We further recommend that soil analyses for micronutrient concentrations should be conducted in future research to determine the levels of these traits and the amounts of minerals or the ability of South African genotypes/cultivars to absorb minerals from the rhizosphere. Modern wheat genotypes have narrow genetic variations and research around the world has advanced towards the introgression of alien genes into modern cultivar and this offers a better alternative option for cultivars with higher mineral variations.

5.3 Suggestions or future work

According to the results of this study, there was considerable variation for QTL analysis in different environments. This indicates that the environment played an important role in the expression of traits. For the consistent QTLs detected in this study, SNP markers associated with them could be informative in increasing the frequency of desirable alleles during early generations of breeding. Moreover, many minor QTLs were detected and these covered much variation for all traits, therefore marker assisted recurrent selection (MARS) will be of great use to further investigate these QTLs and for the development of high-yielding cultivars with improved minerals. The ARC in SA has all the necessary wheat germplasm, technologies and facilities, thus making the study attainable.

5.4 Study contributions

The results of the yield-related traits are currently being drafted for publication purposes. This paper is at the final stages of drafting and will be sent in for publication soon. The results of mineral genetic analyses on doubled haploid lines developed at the ARC-SG will be submitted for the publication as well. Both papers will shed some light on, (i) the existing baseline micronutrient levels, the degree of genetic variation available within the ARC-SG genepool and the genomic regions influencing their genetic variation; and (ii) the relationship between minerals and agronomic traits. This study has contributed, to a certain extent, an understanding of this untapped field in SA but the results from this study call for more consorted research to be done.

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APPEDICES

Appendix I Ethics approval letter

UNISA university of south africa

CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 28/03/2019

NHREC Registration # : REC-170616-051
REC Reference # : 2018/CAES/045
Name : Ms MC Lephuthing
Student #: 61285943

Dear Ms Lephuthing

Decision: Ethics Approval Renewal after First Review from 01/04/2019 to 31/03/2020

Researcher(s): Ms MC Lephuthing
LephuthingM@arc.agric.za

Supervisor (s): Prof TJ Tsilo
TsiloT@arc.agric.za; 058-307-4002

Dr VL Tolmay
TolmayV@arc.agric.za; 058-307-3433

Working title of research:

Characterisation of South African wheat genotypes to improve nutritional quality

Qualification: MSc Life Science

Thank you for the submission of your progress report to the CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is renewed for a one-year period. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 31 March 2020

*The **low risk application** was **reviewed** by the CAES Health Research Ethics Committee on 16 March 2018 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

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Appendix II Genotypes of the Doubled Haploid lines mapping populations and their phenotypic data

Arlington 2018																			
Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn	Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn
TE1	34,13	7,88	9,13	1,05	32,15	10,41	4,82	62,52	62,17	TE42	57,63	10,31	13,75	1,21	25,28	9,52	4,67	72,41	54,63
TE2	*	*	*	*	*	*	*	*	*	TE43	31,75	7,81	9,13	0,74	22,06	9,47	4,37	62,47	71,69
TE3	34,88	8,13	9,50	0,92	25,28	9,71	4,56	71,70	58,58	TE44	37,13	7,38	9,63	0,78	18,85	9,65	4,50	70,99	84,67
TE4	*	*	*	*	*	*	*	*	*	TE45	40,75	9,81	10,63	0,82	16,91	10,19	4,50	50,05	54,15
TE5	18,75	7,56	8,25	0,42	22,19	10,11	4,38	90,48	130,99	TE46	34,50	7,50	8,50	0,83	22,45	9,89	4,51	72,45	86,72
TE6	*	*	*	*	*	*	*	*	*	TE47	35,80	11,00	9,80	1,16	33,78	10,82	4,81	72,46	82,47
TE7	*	*	*	*	*	*	*	*	*	TE48	38,13	9,38	10,63	1,14	28,72	9,65	4,79	66,32	67,97
TE8	28,00	8,00	8,63	0,74	26,23	9,94	4,34	67,85	60,84	TE49	28,13	7,31	7,25	0,55	18,81	9,38	4,16	67,52	70,11
TE9	34,29	7,07	8,57	0,77	21,52	8,97	4,33	66,01	58,21	TE50	53,88	11,19	13,25	1,35	26,74	10,08	4,55	61,52	52,70
TE10	43,88	8,25	9,63	0,85	19,66	9,22	4,27	83,65	88,89	TE51	31,00	6,75	8,75	0,74	23,47	9,45	4,24	59,28	49,28
TE11	*	*	*	*	*	*	*	*	*	TE53	26,75	6,81	7,75	0,88	34,11	10,18	5,02	54,61	58,36
TE12	*	*	*	*	*	*	*	*	*	TE54	24,63	7,88	10,00	0,91	35,35	10,58	4,83	53,42	65,25
TE13	41,50	8,88	10,88	1,42	31,64	10,36	5,47	77,33	62,62	TE55	29,13	8,19	9,88	1,11	36,27	10,48	4,87	67,61	77,22
TE14	35,00	9,63	13,50	0,90	25,81	10,15	4,73	93,49	72,45	TE56	37,88	9,25	11,50	1,12	28,33	9,68	4,56	71,62	58,91
TE15	30,50	8,50	6,50	0,56	29,06	10,98	4,36	111,06	98,86	TE57	28,75	8,31	10,50	0,60	16,39	9,73	4,20	68,13	66,89
TE16	*	*	*	*	*	*	*	*	*	TE58	*	*	*	*	*	*	*	*	*
TE17	33,63	7,00	7,75	0,82	21,84	10,07	4,48	71,15	91,17	TE59	*	*	*	*	*	*	*	*	*
TE18	43,00	9,94	11,63	1,42	34,88	10,17	4,80	64,77	56,97	TE60	38,88	8,63	10,88	0,92	21,66	10,39	4,35	72,90	85,58
TE19	29,25	7,06	8,75	0,72	24,91	9,93	4,39	77,73	90,39	TE61	48,38	9,38	12,13	1,41	29,53				
TE20	*	*	*	*	*	*	*	*	*	TE62	41,13	9,06	12,50	1,43	33,92	10,57	4,79	68,12	65,81
TE21	*	*	*	*	*	*	*	*	*	TE63	20,00	6,42	7,50	0,60	30,84	*	*	*	*
TE22	*	*	*	*	*	*	*	*	*	TE64	38,75	9,94	12,00	1,20	31,93	10,33	4,79	73,83	65,75
TE23	27,00	6,67	7,00	0,67	23,82	9,74	4,26	77,02	78,62	TE66	27,25	8,00	8,88	0,84	29,89	10,30	4,73	61,83	77,06
TE24	38,00	6,88	7,88	0,82	21,11	9,54	4,38	73,84	71,81	TE67	39,38	7,56	11,00	1,36	35,63	9,87	4,75	72,85	65,39
TE25	*	*	*	*	*	*	*	*	*	TE68	36,29	8,43	10,14	1,21	33,26	9,87	4,83	63,54	56,47
TE26	40,88	8,38	10,00	1,09	27,67	9,68	4,54	55,71	71,21	TE69	*	*	*	*	*	*	*	*	*
TE27	*	*	*	*	*	*	*	*	*	TE70	31,50	7,13	8,75	0,74	25,63	9,00	4,37	103,96	70,89
TE28	*	*	*	*	*	*	*	*	*	TE71	16,20	7,20	9,00	0,52	41,26	9,56	4,98	108,47	98,13
TE29	31,57	7,43	9,43	0,77	23,92	9,87	4,46	84,22	93,37	TE73	28,25	6,81	8,38	0,81	27,87	9,63	4,66	67,13	68,48
TE30	28,75	8,19	8,75	0,74	32,90	10,56	5,37	87,28	79,93	TE74	41,38	7,81	8,88	0,84	17,62	9,49	4,34	77,60	87,54
TE32	23,38	7,25	8,38	0,61	26,99	10,30	4,28	84,28	84,13	TE75	46,14	8,71	10,71	1,16	30,07	10,29	4,68	72,28	82,20
TE33	33,75	8,50	9,75	1,00	31,09	10,15	4,60	75,60	76,70	TE76	31,50	8,44	9,63	0,99	27,99	10,57	4,62	87,27	115,77
TE34	24,40	6,80	8,00	0,49	22,35	9,07	4,14	68,28	67,86	TE77	48,88	8,38	11,88	1,26	25,71	10,25	4,40	61,48	60,16
TE35	*	*	*	*	*	*	*	*	*	TE78	*	*	*	*	*	*	*	*	*
TE36	*	*	*	*	*	*	*	*	*	TE79	31,00	8,69	11,13	0,95	30,33	9,93	4,60	63,51	54,99
TE37	36,75	8,25	9,63	0,91	24,66	9,32	4,13	80,05	73,64	TE80	34,50	8,81	11,63	1,05	30,21	9,50	4,77	60,09	58,23
TE38	48,75	10,69	17,88	1,18	30,58	10,01	4,57	70,31	72,67	TE81	19,17	6,17	7,17	0,61	32,66	10,46	4,51	73,03	82,47
TE40	46,50	8,63	11,75	0,86	15,74	9,68	4,33	79,38	57,99	TE83	35,38	9,06	12,13	1,15	30,91	10,14	4,84	46,87	57,36
TE41	29,88	7,25	9,13	0,76	23,61	*	*	*	*	TE84	29,13	6,88	7,50	0,58	18,22	*	*	*	*

Arlington 2018																			
Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn	Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn
TE85	39,00	8,06	10,13	1,15	29,96	10,07	5,14	47,00	78,21	TE123	28,63	7,56	9,25	0,78	25,69	9,72	4,66	63,39	75,62
TE86	*	*	*	*	*	*	*	*	*	TE124	28,75	7,88	8,75	0,49	17,15	8,43	3,79	88,63	98,40
TE87	49,29	8,86	13,43	1,18	22,15	9,36	4,32	55,58	50,65	TE125	26,13	7,63	9,75	0,61	25,17	8,98	4,49	81,77	70,37
TE89	30,13	7,63	8,00	0,84	27,53	9,97	4,16	43,10	61,99	TE126	40,13	7,63	9,75	1,06	26,19	9,62	4,78	69,50	62,73
TE90	*	*	*	*	*	*	*	*	*	TE127	24,50	7,44	8,13	0,70	28,18	10,04	4,79	68,76	63,99
TE91	39,25	8,19	10,63	1,01	23,25	8,88	4,34	52,46	59,39	TE128	28,29	7,71	8,71	0,61	20,51	9,64	4,55	57,06	55,73
TE92	20,25	8,38	9,25	0,59	22,04	9,67	4,53	62,19	96,28	TE129	*	*	*	*	*	*	*	*	*
TE93	24,63	7,13	8,25	0,74	29,52	9,47	4,36	58,86	74,95	TE130	25,00	6,25	8,33	0,36	14,55	*	*	*	*
TE94	34,88	8,69	12,00	1,11	32,51	9,90	4,77	53,67	69,59	TE131	*	*	*	*	*	*	*	*	*
TE95	28,00	7,19	9,88	0,77	31,85	9,95	4,46	53,47	72,06	TE132	34,13	8,44	9,75	0,67	21,44	9,63	4,25	59,61	65,88
TE96	*	*	*	*	*	*	*	*	*	TE133	*	*	*	*	*	*	*	*	*
TE97	26,00	7,50	7,00	0,89	34,84	9,58	4,43	*	*	TE135	23,00	6,25	6,50	0,56	24,67	9,21	4,64	71,77	65,98
TE98	*	*	*	*	*	*	*	*	*	TE136	38,38	9,94	11,00	1,17	24,92	10,04	5,17	56,41	66,78
TE99	29,63	8,75	11,75	0,70	26,18	9,15	4,43	63,16	85,04	TE139	*	*	*	*	*	*	*	*	*
TE101	41,50	8,88	12,25	1,37	32,74	10,11	4,72	56,28	57,42	TE140	37,00	9,50	12,00	0,78	24,17	9,75	5,08	59,02	56,31
TE102	30,25	8,38	9,50	0,75	26,71	10,39	4,38	61,24	79,74	TE141	32,50	7,81	9,50	0,71	21,21	9,03	4,08	78,23	78,87
TE103	32,38	8,25	11,50	0,92	27,94	10,44	4,88	49,48	62,46	TE142	*	*	*	*	*	*	*	*	*
TE104	36,88	8,38	10,25	0,99	30,42	9,20	4,65	50,90	64,04	TE144	36,38	8,69	11,00	1,05	29,65	10,29	4,54	77,33	66,84
TE105	34,88	8,50	10,88	0,96	26,09	9,74	4,59	60,41	62,99	TE145	34,75	8,13	10,00	1,06	35,73	11,11	5,00	51,52	66,74
TE106	31,00	8,00	10,17	0,67	20,61	10,20	4,27	55,52	45,86	TE146	17,71	14,21	6,57	0,33	19,51	9,25	3,53	54,40	62,44
TE109	*	*	*	*	*	*	*	*	*	TE146	17,71	14,21	6,57	0,33	19,51	9,25	3,53	54,40	62,44
TE111	37,00	9,25	11,00	1,22	27,31	9,74	4,47	60,16	56,08	TE147	*	*	*	*	*	*	*	*	*
TE112	46,00	10,19	13,38	1,61	35,21	10,86	5,07	73,26	63,29	TE148	28,63	7,56	9,75	0,93	32,11	10,16	4,55	52,65	61,01
TE113	34,25	7,31	9,50	1,03	29,04	9,53	4,70	54,32	56,47	TE149	27,88	7,75	11,25	0,61	21,41	9,29	4,24	58,78	85,67
TE114	*	*	*	*	*	*	*	*	*	TE150	35,38	8,06	11,00	0,88	26,49	9,75	4,91	50,35	66,17
TE115	36,00	7,50	10,17	0,99	27,98	9,24	4,33	64,49	67,83	TE151	34,50	10,38	13,00	1,00	28,75	10,63	5,03	46,53	61,53
TE116	40,63	8,50	9,75	1,04	18,27	9,64	4,35	74,50	88,01	TE152	25,33	6,25	8,83	0,68	25,29	9,53	4,55	61,81	96,07
TE118	*	*	*	*	*	*	*	*	*	TE153	*	*	*	*	*	*	*	*	*
TE119	32,00	8,00	10,50	1,07	33,72	10,08	4,62	80,00	65,03	TE155	24,25	7,50	9,75	0,59	24,97	9,20	4,18	80,29	97,65
TE120	*	*	*	*	*	*	*	*	*	TE156	33,67	6,83	9,17	0,64	20,57	9,45	4,17	46,51	73,33
TE122	*	*	*	*	*	*	*	*	*	TE158	*	*	*	*	*	*	*	*	*

Bethlehem 2018																			
Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn	Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn
TE1	41,75	10,69	16,38	1,09	28,37	8,96	3,80	*	*	TE42	41,75	10,44	15,63	1,39	33,51	*	*	*	*
TE2	17,38	10,25	13,88	0,49	33,66			43,55	35,87	TE43	51,50	11,94	18,38	1,48	29,46	8,27	4,52	33,43	29,71
TE3	54,13	9,75	17,00	2,18	38,63	9,25	5,09	37,60	28,27	TE44	59,00	10,75	18,75	2,15	36,65	*	*	*	*
TE4	70,75	10,94	21,38	2,98	41,07	8,67	5,09	42,91	30,26	TE45	44,50	12,13	16,25	1,93	43,72	*	*	*	*
TE5	29,63	11,44	17,25	0,78	26,58	8,27	3,90	45,16	43,98	TE46	40,63	10,81	17,38	1,54	37,66	*	*	*	*
TE6	53,13	12,81	18,75	2,52	48,13	9,22	5,28	34,17	37,74	TE47	32,63	10,38	15,63	1,43	45,15	*	*	*	*
TE7	59,38	13,50	19,38	2,38	10,01	8,78	4,95	50,52	37,12	TE48	46,50	12,13	17,13	2,02	42,70	*	*	*	*
TE8	47,25	13,13	18,13	2,04	44,27	9,37	5,03	70,00	51,72	TE49	56,50	12,13	18,38	1,81	31,87	8,16	4,46	*	*
TE9	*	*	*	*	*	*	*	*	*	TE50	45,88	12,19	17,75	1,72	37,81	8,79	4,62	*	*
TE10	*	*	*	*	*	*	*	*	*	TE51	60,75	12,06	18,38	2,03	34,43	8,27	4,51	41,59	30,91
TE11	*	*	*	*	*	*	*	*	*	TE53	52,25	12,00	16,88	2,74	52,62	9,92	5,30	45,29	35,19
TE12	52,50	12,44	19,13	2,03	38,34	9,25	4,58	44,07	35,27	TE54	41,00	10,19	17,38	2,01	47,64	9,35	5,16	*	*
TE13	27,86	9,25	13,25	0,47	19,37	*	*	*	*	TE55	45,75	10,31	16,38	2,19	48,87	9,70	5,08	37,71	30,26
TE14	42,88	10,69	17,38	1,55	37,47	8,39	4,77	36,49	39,16	TE56	43,13	10,63	16,25	1,12	29,62	8,67	4,27	*	*
TE15	44,13	10,06	17,25	1,74	40,48	9,36	4,44			TE57	45,25	10,56	16,38	2,02	45,29	9,26	5,09	*	*
TE16	27,25	8,94	14,50	0,87	34,35	9,29	4,20	34,49	29,75	TE58	6,00	9,25	10,50	0,12	19,87	*	*	*	*
TE17	53,75	11,69	19,50	2,45	48,92	9,42	5,34	46,54	40,58	TE59	46,63	10,94	17,13	1,69	38,36	9,05	4,81	*	*
TE18	33,88	10,88	16,00	0,87	27,76	*	*	*	*	TE60	35,50	9,00	14,63	1,46	41,82	*	*	*	*
TE19	31,00	8,44	15,13	1,11	37,02	*	*	40,72	34,75	TE61	48,50	10,69	18,63	1,56	33,38	8,64	4,24	47,49	45,68
TE20	49,63	10,31	18,25	1,77	37,39	*	*	52,35	37,89	TE62	49,63	10,88	18,88	2,07	43,02	9,91	4,49	52,31	35,50
TE21	53,13	11,25	17,88	2,13	40,58	9,15	4,65	*	*	TE63	48,00	10,94	17,63	2,16	45,69	10,38	5,08	56,17	34,48
TE22	59,38	11,56	23,88	2,34	39,98	8,63	5,15	54,34	33,68	TE64	64,13	12,75	20,50	1,98	32,26	9,27	4,38	52,01	44,48
TE23	41,88	10,25	16,50	1,68	38,12	9,15	4,69	32,11	26,54	TE66	41,13	10,75	15,63	1,24	30,98	9,25	4,12	*	*
TE24	58,00	11,38	19,75	2,15	39,88	8,87	5,04	38,18	32,40	TE67	61,88	11,63	21,50	2,06	35,61	9,19	4,66	*	*
TE25	52,50	10,88	19,25	2,04	38,98	8,69	4,64	31,98	31,79	TE68	65,13	13,75	21,50	2,84	42,03	8,95	4,94	47,68	29,99
TE26	43,63	11,56	19,38	1,64	33,98	8,64	4,75	28,12	27,23	TE69	49,25	11,94	17,88	2,20	43,82	9,69	4,78	42,55	37,73
TE27	42,13	10,75	19,13	1,76	41,03			33,73	34,05	TE70	58,00	11,31	18,13	1,76	32,99	8,09	4,75	59,38	50,33
TE28	48,63	13,25	19,88	2,14	43,45	8,72	4,89	36,62	33,80	TE71	41,13	10,56	15,88	1,93	48,46	9,09	5,15	*	*
TE29	56,13	14,31	20,50	2,67	47,11	8,89	5,02	46,35	30,14	TE73	52,50	11,38	17,38	2,10	42,04	8,86	5,07	52,94	38,64
TE30	60,25	13,63	21,13	2,27	39,45	9,36	5,20	49,71	39,68	TE74	34,25	8,63	14,50	1,01	30,44	8,48	4,48	34,03	32,88
TE32	43,00	10,31	17,25	1,86	45,17	9,13	4,48	*	*	TE75	46,00	10,19	15,00	1,69	37,18	*	*	*	*
TE33	51,63	12,44	18,25	2,49	49,77	8,99	4,78	*	*	TE76	59,75	13,06	19,38	2,53	43,13	9,46	4,80	*	*
TE34	41,13	10,00	16,63	1,29	31,49	9,17	5,11	*	*	TE77	51,38	12,13	20,00	1,31	26,87	8,72	4,19	46,22	43,24
TE35	63,63	13,06	20,13	2,00	31,47	9,29	4,55	43,63	37,60	TE78	44,75	10,69	16,38	1,75	39,13	9,09	4,72	44,43	35,27
TE36	43,38	11,50	18,13	1,58	35,97	9,16	4,87	41,46	36,63	TE79	50,50	11,69	17,88	1,75	36,82	8,81	4,69	*	*
TE37	53,13	11,38	18,88	1,66	30,84	8,31	4,31	49,33	43,78	TE80	43,00	10,94	15,63	1,85	42,65	9,15	4,90	34,20	47,69
TE38	*	*	*	*	*	*	*	*	*	TE81	*	*	*	*	*	*	*	*	*
TE40	56,88	12,25	21,50	2,36	42,18	9,08	4,83	51,06	34,36	TE83	11,00	9,00	11,25	0,28	25,09	*	*	*	*
TE41	52,88	10,75	17,63	1,95	36,66	8,66	4,65	*	*	TE84	45,88	10,19	17,88	1,14	25,51	8,09	4,00	*	*

Bethlehem 2018																			
Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn	Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn
TE85	24,50	9,63	12,50	0,70	28,19	9,16	4,46	*	*	TE123	55,63	12,13	18,88	1,82	32,78	8,95	4,88	22,88	37,87
TE86	42,00	9,94	17,13	1,46	35,04	*	*	*	*	TE124	43,75	11,38	16,88	1,20	27,09	9,33	4,70	30,15	47,38
TE87	44,63	9,88	17,75	1,65	37,51	8,88	5,25	27,76	48,39	TE125	53,50	13,50	21,25	1,60	32,48	8,69	4,49	36,52	41,32
TE89	45,38	11,94	18,38		37,80	9,55	4,71	37,35	35,33	TE126	32,38	10,50	16,00	0,66	19,95	8,05	4,43	*	*
TE90	45,57	11,00	16,29	1,70	39,25	*	*	*	*	TE127	44,75	11,56	17,13	1,74	37,78	8,54	4,50	52,18	36,42
TE91	53,38	11,25	20,13	1,52	28,74	8,39	4,46	*	*	TE128	49,75	11,56	18,75	2,06	42,35	8,39	4,19		
TE92	53,50	11,25	18,00	1,82	35,98	9,15	4,67	*	*	TE129	47,50	10,06	16,50	1,76	37,35	9,03	4,79	61,32	47,03
TE93	53,75	11,63	17,50	1,50	28,62	8,96	4,42	37,07	49,26	TE130	55,38	10,50	17,63	2,14	38,71	8,61	4,45	65,61	39,18
TE94	41,63	12,00	21,50	1,30	32,16	*	*	*	*	TE131	48,13	11,31	18,50	2,05	42,63	9,36	4,89	67,23	51,80
TE95	57,00	13,44	20,88	1,77	33,21	8,87	4,62	34,43	38,56	TE132	47,38	11,88	18,75	1,89	40,06	8,88	4,67	*	*
TE96	49,38	10,50	16,75	1,87	39,23	8,11	5,01	23,50	42,13	TE133	58,88	13,13	21,50	2,08	34,53	9,10	4,91	*	*
TE97	47,38	12,19	17,50	2,06	44,85	9,07	4,73			TE135	42,13	10,25	16,88	1,99	46,52	9,32	4,79	60,31	37,90
TE98	67,25	13,69	21,13	3,14	45,03	9,73	4,86	39,03	57,54	TE136	47,75	12,06	17,25	1,75	34,43	8,80	4,84	56,61	30,67
TE99	49,25	11,94	17,88	1,44	31,25	8,75	4,19	41,80	46,73	TE139	57,75	12,38	19,63	1,55	27,82	8,40	5,19	68,93	37,52
TE101	50,00	11,94	18,25	1,91	36,01	10,06	4,75	30,84	40,88	TE140	55,88	11,69	18,88	2,32	43,24	9,64	4,42	59,12	39,19
TE102	44,75	11,56	17,00	1,66	39,18	*	*	*	*	TE141	44,88	10,69	16,50	1,80	42,09	8,23	4,31	*	*
TE103	37,00	9,94	16,38	1,48	43,46	9,18	5,19	57,12	53,44	TE142	54,63	10,44	17,63	2,22	41,13	8,90	4,95	66,40	41,51
TE104	48,88	11,81	18,63	1,44	28,48	8,31	4,32	80,68	58,18	TE144	48,00	11,56	17,50	1,95	41,44	9,19	4,88	59,21	39,39
TE105	42,75	11,13	16,88	2,04	46,27	9,12	5,09	47,53	59,74	TE145	51,88	10,94	17,63	2,31	42,23	8,46	4,75	51,66	29,22
TE106	53,38	11,69	20,13	2,00	37,46	9,00	4,57	*	*	TE146	41,63	10,69	16,38	1,51	35,81	8,50	4,82	52,62	37,01
TE109	27,00	10,19	16,50	0,29	11,32	*	*	*	*	TE147	51,13	11,94	18,13	1,90	37,75	10,16	4,72	49,75	34,89
TE111	56,13	12,38	18,63	2,38	42,75	9,30	4,81	86,44	60,27	TE148	51,88	11,56	18,75	2,13	41,96	9,52	4,53	46,29	34,67
TE112	57,50	12,81	21,38	2,43	41,15	9,14	4,80	61,18	51,34	TE149	40,63	10,63	17,75	1,00	25,19	9,83	4,96	46,30	33,67
TE113	68,75	12,44	20,88	2,02	29,76	8,77	4,11	66,03	58,16	TE150	62,63	11,81	20,00	2,46	39,41	9,46	4,77	49,98	51,79
TE114	55,63	11,56	21,75	2,32	43,13	8,72	4,67	*	*	TE151	48,00	13,19	18,50	1,41	27,42	8,50	4,32	61,72	47,69
TE115	69,50	13,63	22,38	2,85	39,69	8,98	4,64	68,04	59,32	TE152	56,25	10,38	18,00	1,57	29,81	9,16	4,67	50,90	43,30
TE116	55,25	12,38	19,63	1,96	34,62	8,37	4,63	39,14	34,86	TE153	54,63	12,56	17,63	2,64	48,81	9,55	4,33	68,43	37,34
TE118	55,38	12,50	20,63	1,80	30,95	9,07	4,78	*	*	TE155	62,13	11,13	18,25	2,02	33,92	8,49	4,43	70,14	48,47
TE119	*	*	*	*	*	*	*	*	*	TE156	*	*	*	*	*	*	*	*	*
TE120	51,63	11,13	16,63	1,95	38,51	9,65	4,63	10,02	35,52	TE158	61,63	11,56	19,00	3,20	53,87	10,14	5,03	82,37	54,23
TE122	47,13	12,31	16,88	1,70	36,46	9,55	4,12												

Harrismith 2018																			
Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn	Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn
TE1	51,75	10,25	17,00	1,65	30,84	*	*	*	*	TE42	56,00	11,00	18,13	1,59	28,75	*	*	*	*
TE2	40,50	10,38	16,88	1,45	25,82	10,28	5,03	64,70	70,35	TE43	56,00	10,63	19,13	1,44	25,74	*	*	*	*
TE3	39,13	9,63	15,63	1,06	21,56	9,96	4,95	120,05	31,32	TE44	42,88	9,38	14,75	1,57	31,57	9,93	4,78	65,52	41,49
TE4	46,25	10,88	18,88	1,31	22,43	9,80	4,94	64,45	56,87	TE45	36,25	10,88	15,13	1,30	28,84	*	*	*	*
TE5	42,88	10,00	15,38	1,57	37,99	10,12	4,72	65,04	36,06	TE46	31,25	9,00	15,25	0,77	24,86	10,39	5,12	63,28	36,26
TE6	33,38	10,63	15,13	1,19	26,95	10,50	5,21	55,20	42,92	TE47	*	*	*	*	*	*	*	*	*
TE7	42,63	10,63	17,13	1,52	24,95	*	*	*	*	TE48	48,00	12,13	18,00	1,30	26,96	9,66	4,75	77,43	42,77
TE8	43,88	10,38	17,13	1,95	44,04	*	*	*	*	TE49	45,25	12,44	19,75	1,39	30,19	9,20	4,60	66,07	51,80
TE9	48,50	9,00	17,13	1,19	21,63	*	*	*	*	TE50	44,75	10,19	17,00	0,88	19,47	9,02	4,26	75,15	38,72
TE10	57,75	10,38	19,25	1,57	29,09	9,17	4,35	73,10	36,66	TE51	*	*	*	*	*	*	*	*	*
TE11	40,13	10,75	16,25	1,57	25,28	*	*	*	*	TE53	31,88	10,13	13,75	0,74	18,13	*	*	*	*
TE12	46,63	10,50	17,75	1,44	22,65	10,72	5,25	59,12	29,88	TE54	38,38	11,19	17,38	1,74	45,34	10,74	5,07	68,42	38,58
TE13	39,13	9,75	13,38	1,46	36,67	*	*	*	*	TE55	38,75	9,94	15,50	1,47	36,96	10,85	4,86	76,03	38,85
TE14	50,50	8,75	15,88	1,50	30,37	9,26	4,44	71,63	28,87	TE56	42,25	11,81	17,63	0,97	22,89	8,87	4,00	70,21	33,40
TE15	*	*	*	*	*	*	*	*	*	TE57	40,50	10,50	16,13	1,20	21,33	*	*	*	*
TE16	25,00	10,50	18,00	0,53	20,69	*	*	*	*	TE58	*	*	*	*	*	*	*	*	*
TE17	44,00	9,88	16,00	1,87	41,31	10,34	4,97	60,99	35,26	TE59	56,38	12,88	17,88	1,83	18,33	*	*	*	*
TE18	37,38	10,00	13,50	1,21	25,23	*	*	*	*	TE60	29,75	10,06	15,25	0,72	24,09	*	*	*	*
TE19	*	*	*	*	*	*	*	*	*	TE61	50,13	12,00	19,00	1,20	23,82	8,88	3,79	77,94	38,15
TE20	47,50	9,88	19,25	1,14	20,15	9,85	4,42	58,08	42,45	TE62	34,75	11,00	14,75	1,37	38,67	9,94	4,95	70,12	40,98
TE21	34,88	10,75	15,38	1,43	41,27	*	*	*	*	TE63	26,25	10,13	13,75	0,96	35,05	10,43	4,73	81,00	42,27
TE22	31,88	10,38	16,25	0,91	21,23	10,31	4,74	69,46	45,11	TE64	44,38	13,63	18,75	1,22	22,94	9,70	4,40	71,26	47,37
TE23	42,75	9,50	15,38	1,59	38,38	10,05	4,96	56,21	32,89	TE66	34,25	11,06	16,38	0,76	18,66	*	*	*	*
TE24	34,29	10,86	18,86	0,95	22,52	10,03	4,86	73,43	51,69	TE67	52,13	11,00	19,13	1,52	32,01	10,10	4,23	90,47	44,40
TE25	40,75	9,88	16,88	1,53	38,51	9,99	5,02	62,21	50,13	TE68	56,25	12,06	17,50	2,08	37,63	10,09	4,82	62,19	29,06
TE26	*	*	*	*	*	*	*	*	*	TE69	52,25	12,38	17,13	2,25	43,91	10,93	5,05	71,84	37,00
TE27	48,38	10,50	18,63	1,63	33,87	*	*	*	*	TE70	47,88	11,56	17,38	1,44	29,22	9,66	5,09	83,51	39,99
TE28	24,50	9,00	13,50	0,62	14,71	9,82	4,59	57,41	29,31	TE71	39,13	11,75	16,88	1,74	45,41	*	*	*	*
TE29	44,38	11,00	17,25	1,73	36,37	10,26	4,95	61,79	42,58	TE73	35,13	8,69	14,50	1,19	32,87	9,80	4,70	87,95	45,69
TE30	50,00	10,71	19,29	1,64	32,06	9,87	4,75	54,10	33,23	TE74	40,00	11,25	16,88	1,49	25,67	10,32	5,17	81,49	53,02
TE32	37,00	9,25	15,63	1,63	43,88	*	*	*	*	TE75	52,75	11,75	18,00	1,97	36,51	*	*	*	*
TE33	38,13	10,00	14,50	1,23	23,28	*	*	*	*	TE76	49,63	13,25	19,75	1,65	24,06	10,53	4,57	51,92	28,13
TE34	*	*	*	*	*	*	*	*	*	TE77	38,63	9,94	18,38	0,81	22,09	*	*	*	*
TE35	34,63	8,38	12,75	0,95	18,59	9,35	4,67	60,77	35,01	TE78	24,50	9,44	14,25	0,32	12,43	9,69	4,10	81,74	43,38
TE36	33,75	8,88	14,13	0,98	23,06	10,02	4,87	63,95	52,86	TE79	*	*	*	*	*	*	*	*	*
TE37	40,50	9,63	15,50	1,40	34,42	9,81	4,57	76,06	36,83	TE80	28,14	9,71	13,29	0,46	15,07	*	*	*	*
TE38	45,63	10,38	19,25	1,44	31,65	*	*	*	*	TE81	36,75	11,69	15,75	1,18	34,36	9,59	4,62	92,55	54,16
TE40	51,75	10,13	17,38	1,88	33,25	10,07	4,80	61,31	32,96	TE83	*	*	*	*	*	*	*	*	*
TE41	50,38	8,50	16,13	1,39	27,54	*	*	*	*	TE84	40,25	9,63	14,63	0,95	22,66	9,36	4,15	67,91	47,31

Harrismith 2018																			
Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn	Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn
TE85	37,38	11,63	17,75	1,05	28,45	*	*	*	*	TE123	45,50	10,13	17,63	1,15	25,15	9,42	4,24	70,76	48,83
TE86	36,63	10,75	16,38	1,26	33,14	*	*	*	*	TE124	40,25	9,88	15,00	1,00	18,98	9,71	5,00	72,54	51,41
TE87	57,13	11,50	21,75	1,65	29,75	9,48	4,65	95,31	33,11	TE125	58,63	11,00	18,13	1,75	30,46	*	*	*	*
TE89	38,88	11,44	17,00	0,92	16,69	10,54	4,72	63,58	48,39	TE126	44,50	10,44	16,00	1,09	23,23	9,67	4,71	63,92	43,06
TE90	36,38	9,06	15,63	1,11	27,75	*	*	*	*	TE127	40,00	10,75	15,75	1,15	22,59	9,89	4,78	44,60	33,54
TE91	37,00	10,25	16,13	0,93	25,67	9,06	4,27	55,19	33,04	TE128	43,75	10,63	18,00	1,18	19,03	9,70	4,34	59,65	39,10
TE92	39,75	9,00	13,75	0,90	20,91	10,32	5,10	53,87	38,92	TE129	39,00	9,75	13,63	1,28	33,49	10,22	4,55	77,32	56,21
TE93	49,25	9,50	16,50	1,04	21,75	9,09	4,03	97,45	47,70	TE130	37,43	7,21	14,00	0,82	21,99	9,32	4,24	63,88	45,81
TE94	40,25	11,31	20,13	0,91	22,66	*	*	*	*	TE131	43,00	11,19	15,25	1,28	29,58	9,77	4,61	72,02	51,03
TE95	37,38	11,50	16,38	0,99	26,69	9,67	4,36	56,96	39,78	TE132	41,50	11,75	16,25	1,16	29,56	9,51	4,44	88,40	37,14
TE96	42,75	10,38	16,50	1,16	26,86	9,59	4,30	54,54	40,85	TE133	41,50	12,06	16,38	1,20	25,97	9,35	4,62	63,58	45,03
TE97	30,13	9,50	12,00	1,21	39,16	*	*	*	*	TE135	*	*	*	*	*	*	*	*	*
TE99	46,25	10,19	17,50	1,51	33,19	9,50	4,54	55,56	44,13	TE136	*	*	*	*	*	*	*	*	*
TE100	*	*	*	*	*	*	*	*	*	TE139	53,00	12,63	20,13	1,74	33,61	9,34	4,52	84,16	44,85
TE101	44,88	9,75	15,50	1,21	23,62	*	*	*	*	TE140	47,00	10,25	16,50	1,69	26,35	9,90	4,82	68,29	31,75
TE102	49,50	11,38	17,75	1,86	36,15	10,48	4,78	63,48	41,13	TE141	*	*	*	*	*	*	*	*	*
TE103	42,63	11,13	17,00	1,28	30,29	10,24	4,70	77,25	65,26	TE142	51,00	11,00	15,38	1,70	22,04	10,30	5,15	90,47	47,24
TE104	43,25	11,63	17,00	1,43	34,29	*	*	*	*	TE144	41,63	11,44	16,25	0,95	21,04				
TE105	40,38	10,63	16,25	1,68	43,79	9,85	4,97	64,18	51,87	TE145	51,88	10,88	16,63	1,55	21,62	11,58	4,90	85,23	49,53
TE106	43,25	11,25	17,13	1,14	20,32	10,51	4,54	61,13	36,32	TE146	48,00	9,44	16,25	1,43	27,81	9,87	4,21	58,87	47,52
TE109	44,63	11,69	18,50	0,98	24,05	*	*	*	*	TE147	37,25	10,94	16,38	1,14	25,46	10,34	4,81	72,80	47,79
TE111	51,50	11,63	16,88	1,77	35,57	*	*	*	*	TE148	*	*	*	*	*	*	*	*	*
TE112	*	*	*	*	*	*	*	*	*	TE149	45,50	10,50	18,13	1,00	14,76	9,69	4,32	78,69	43,34
TE113	28,25	6,63	12,38	0,31	11,04	7,54	3,47	67,30	42,25	TE150	37,75	8,63	12,88	0,94	24,41	9,77	4,43	69,41	57,50
TE114	40,75	8,63	17,00	1,07	25,94	9,55	4,43	52,12	33,35	TE151	25,88	11,94	17,25	0,41	11,94	9,87	4,35	60,29	36,00
TE115	42,88	10,44	16,25	0,73	15,84	*	*	*	*	TE152	47,50	8,75	17,38	1,17	25,23	9,25	4,44	68,92	53,96
TE116	*	*	*	*	*	*	*	*	*	TE153	50,88	10,63	18,13	1,37	20,79	*	*	*	*
TE118	43,88	10,88	18,50	0,81	15,08	*	*	*	*	TE155	*	*	*	*	*	*	*	*	*
TE119	*	*	*	*	*	*	*	*	*	TE156	*	*	*	*	*	*	*	*	*
TE120	*	*	*	*	*	*	*	*	*	TE158	*	*	*	*	*	*	*	*	*
TE122	33,75	11,63	15,13	1,20	36,41	*	*	*	*										

Bethlehem 2019																		
Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn	Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn
TE1	36,75	9,63	13,50	1,57	43,62	10,12	4,98	130,71	52,38	TE43	45,88	9,81	15,50	1,91	45,26	9,43	5,27	52,17	46,72
TE2	*	*	*	*	*	*	*	*	*	TE44	38,88	8,38	13,75	1,65	44,83	9,35	5,19	54,55	45,48
TE3	46,38	10,50	14,75	1,85	40,30	9,59	5,07	*	*	TE45	30,88	9,19	12,00	1,27	44,45	9,55	4,97	55,66	49,47
TE4	56,75	10,13	15,75	2,27	40,12	9,34	5,23	137,17	81,15	TE46	35,13	8,19	13,00	1,42	43,08	9,92	5,05	57,98	42,82
TE5	*	*	*	*	*	*	*	*	*	TE47	25,00	9,42	9,50	0,93	38,21	10,13	4,49	63,61	51,73
TE6	43,75	10,25	17,00	1,99	48,12	9,81	5,37	106,96	64,42	TE48	33,25	8,44	13,13	1,16	35,32	8,91	4,45	59,95	51,69
TE7	42,00	11,75	16,25	1,87	47,24	9,64	5,35	87,84	61,95	TE49	46,00	9,75	15,63	1,84	41,99	8,90	5,02	60,08	54,17
TE8	47,38	10,63	17,25	2,08	43,45	9,47	4,90	79,96	51,01	TE50	45,25	10,25	14,13	2,10	47,76	9,50	5,47	55,59	46,33
TE9	42,25	8,94	15,75	1,39	33,23	8,52	4,77	63,13	43,71	TE51	42,00	8,13	14,50	1,52	41,08	9,09	5,01	54,79	43,83
TE10	56,38	11,44	20,00	2,31	43,53	9,04	5,08	80,98	44,69	TE53	41,38	8,38	13,75	1,87	44,98	9,09	5,06	51,18	44,40
TE11	51,50	12,63	18,50	2,41	49,27	10,56	5,58	74,34	45,08	TE54	25,00	8,38	11,75	0,97	39,81	9,92	4,89	67,80	51,31
TE12	34,50	9,50	14,50	1,42	43,34	10,07	5,07	76,05	51,39	TE55	35,13	9,50	13,13	1,49	43,02	10,65	4,80	80,29	55,00
TE13	*	*	*	*	*	*	*	*	*	TE56	42,75	10,50	15,25	1,57	38,07	9,63	4,87	71,24	49,98
TE14	*	*	*	*	*	*	*	*	*	TE57	47,75	10,75	15,00	1,99	43,17	9,97	5,02	81,53	45,79
TE15	36,63	8,75	14,13	1,51	41,92	9,97	5,08	70,69	51,54	TE58	*	*	*	*	*	*	*	*	*
TE16	31,75	7,44	13,63	1,67	45,59	9,43	4,97	54,54	41,10	TE59	31,00	8,44	11,50	1,40	45,83	9,79	5,12	85,46	66,54
TE17	37,75	8,38	11,75	1,84	50,90	9,70	5,49	53,25	39,61	TE60	*	*	*	*	*	*	*	*	*
TE18	*	*	*	*	*	*	*	*	*	TE61	45,75	10,25	17,63	1,94	44,22	9,94	5,03	76,30	55,76
TE19	28,13	7,44	11,38	1,25	44,88	9,84	5,03	52,42	40,32	TE62	55,50	10,19	16,38	2,43	46,45	10,31	5,18	94,25	50,77
TE20	38,38	8,56	15,00	1,53	45,47	9,12	5,53	61,98	50,39	TE63	*	*	*	*	*	*	*	*	*
TE21	*	*	*	*	*	*	*	*	*	TE64	50,75	10,69	16,00	1,74	34,87	9,49	4,46	*	*
TE22	39,00	9,88	16,50	1,49	38,13	9,20	4,54	65,57	44,74	TE66	21,71	7,86	9,86	0,77	36,12	9,95	4,65	76,51	67,47
TE23	37,63	9,00	13,75	1,66	45,47	10,06	5,18	61,45	42,49	TE67	47,50	9,00	16,50	2,01	43,16	9,67	5,00	75,84	42,86
TE24	44,88	9,69	16,25	1,69	37,91	9,13	4,71	65,71	50,91	TE68	45,00	10,00	14,75	1,92	43,80	9,83	5,26	73,65	47,91
TE25	47,88	9,13	14,25	1,76	38,29	9,72	5,06	73,77	55,15	TE69	41,88	9,06	14,25	1,85	44,94	10,18	5,14	70,80	48,86
TE26	33,75	8,63	12,13	1,34	40,82	9,51	5,01	69,32	63,76	TE70	52,38	9,19	16,00	2,11	43,12	9,13	5,31	60,55	45,83
TE27	34,25	7,81	12,63	1,47	44,27	9,62	5,04	66,39	53,69	TE71	36,88	7,69	13,88	1,70	46,91	8,84	5,45	47,82	42,96
TE28	41,13	10,25	14,50	1,77	44,70	10,26	5,36	79,45	51,80	TE73	35,88	7,00	11,88	1,39	39,28	9,05	5,03	60,68	40,29
TE29	36,38	10,81	15,75	1,89	51,32	10,25	5,54	62,55	45,73	TE74	30,63	7,25	12,00	1,31	42,28	9,28	5,04	76,42	58,68
TE30	42,25	11,00	18,38	1,79	42,33	9,61	5,09	71,14	54,14	TE75	42,88	9,25	14,88	1,62	38,84	9,25	4,69	69,92	48,32
TE32	38,63	9,38	16,25	1,68	46,08	9,51	4,88	66,54	55,50	TE76	38,13	10,13	14,50	1,53	41,31	10,06	4,86	67,28	51,00
TE33	37,25	10,19	14,63	1,74	45,23	10,26	5,18	79,01	61,17	TE77	33,50	7,94	13,63	1,06	33,13	9,88	4,38	63,74	47,58
TE34	53,38	11,38	19,88	2,32	46,37	9,36	5,25	78,37	57,10	TE78	43,25	9,06	13,50	2,00	47,77	9,82	5,01	77,20	45,56
TE35	*	*	*	*	*	*	*	*	*	TE79	42,13	8,69	14,00	1,89	46,15	9,30	5,23	61,76	39,41
TE36	42,75	9,13	13,63	1,74	43,84	9,94	5,20	69,85	58,60	TE80	46,00	9,69	15,00	2,17	49,79	9,60	5,51	56,81	45,65
TE37	41,13	8,69	14,13	1,46	40,34	9,39	4,93	72,39	59,78	TE81	42,00	9,00	15,75	2,00	47,69	10,10	5,20	63,44	51,09
TE38	*	*	*	*	*	*	*	*	*	TE83	28,50	7,69	10,25	1,32	46,22	9,58	5,37	73,84	48,59
TE40	57,25	9,75	17,63	2,33	43,24	9,19	5,17	57,71	42,02	TE84	*	*	*	*	*	*	*	*	*
TE41	*	*	*	*	*	*	*	*	*	TE85	27,13	8,25	9,88	0,89	35,12	9,23	4,65	67,73	45,50

Bethlehem 2019																			
Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn	Genotype	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe	Zn
TE86	38,63	8,13	14,25	1,48	40,21	9,21	4,77	65,91	41,69	TE123	48,00	9,63	14,38	1,84	41,06	10,01	5,12	46,06	42,63
TE87	38,38	7,75	14,63	1,45	38,44	9,24	4,74	78,64	43,96	TE124	43,13	10,00	14,75	1,44	33,78	9,18	4,79	79,57	55,81
TE89	30,13	8,63	11,50	1,21	40,62	10,89	4,95	69,64	39,18	TE125	43,88	10,25	14,88	1,53	39,95	9,58	4,47	62,77	42,09
TE90	36,00	8,94	15,13	1,30	40,21	9,52	4,97	55,00	50,30	TE126	41,75	8,81	15,13	1,75	42,23	9,72	5,05	68,66	50,69
TE91	36,00	8,31	14,25	1,17	34,67	8,80	4,68	59,02	41,86	TE127	42,50	9,63	14,25	1,86	42,09	10,16	5,16	56,81	44,10
TE92	*	*	*	*	*	*	*	*	*	TE128	33,63	8,44	13,38	1,27	40,68	9,58	5,00	86,60	46,86
TE93	40,50	8,88	15,50	1,47	37,68	9,52	4,84	58,94	45,30	TE129	43,13	9,63	12,88	1,75	42,77	10,70	5,31	79,11	54,09
TE94	41,13	12,06	18,00	1,40	39,36	9,65	4,62	61,18	45,53	TE130	46,38	9,31	15,13	1,66	40,22	9,84	5,04	64,12	44,62
TE95	33,00	10,44	16,00	1,40	39,88	9,88	5,05	55,58	50,54	TE131	32,75	9,19	11,50	1,48	46,45	9,90	5,39	86,10	64,16
TE96	39,13	9,50	13,63	1,61	43,78	9,69	5,43	64,42	40,33	TE132	38,63	10,56	16,25	1,64	44,09	9,55	5,16	68,35	45,87
TE97	*	*	*	*	*	9,69	4,94	*	*	TE133	37,38	9,94	13,00	1,45	41,25	9,85	5,21	93,19	61,20
TE98	45,13	10,38	17,38	1,66	40,04	9,59	4,75	63,83	44,68	TE135	40,50	8,94	14,00	1,73	42,72	9,17	5,16	67,60	48,11
TE99	41,38	10,56	16,88	1,60	39,98	9,63	4,91	61,24	49,30	TE136	33,63	10,31	14,50	1,44	44,57	9,61	5,19	82,65	48,51
TE101	51,25	10,13	16,50	2,12	44,29	10,42	4,91	67,98	43,39	TE139	37,63	11,69	18,63	1,43	39,31	9,54	4,76	58,57	48,38
TE102	42,75	9,88	15,38	1,92	47,33	10,10	5,18	60,45	49,57	TE140	34,13	10,06	17,38	1,56	46,76	9,41	5,18	49,04	47,56
TE103	*	*	*	*	*	*	*	*	*	TE141	36,13	10,38	13,50	1,67	50,58	9,98	5,35	72,08	59,50
TE104	47,25	10,06	16,38	2,06	43,17	9,87	5,14	45,50	37,59	TE142	*	*	*	*	*	*	*	*	*
TE105	46,00	9,44	15,50	2,21	51,02	9,25	5,48	53,82	43,14	TE144	44,75	10,75	16,38	2,19	48,82	9,19	5,29	52,77	47,11
TE106	37,13	7,69	14,13	1,69	46,89	9,98	5,09	50,89	37,97	TE145	48,75	10,31	17,50	2,39	51,02	10,79	5,17	48,78	36,72
TE109	*	*	*	*	*	*	*	*	*	TE146	36,25	10,31	15,88	1,43	41,24	10,48	4,87	59,15	50,98
TE111	51,75	9,19	14,13	2,26	44,52	10,42	5,17	46,22	40,33	TE147	32,63	10,38	16,38	1,41	45,36	9,85	5,10	65,43	51,78
TE112	37,13	9,63	14,88	1,60	43,84	10,33	5,04	62,19	42,55	TE148	39,75	9,88	16,13	1,79	47,26	10,11	5,04	66,85	48,69
TE113	40,38	8,06	13,13	1,55	38,93	9,20	4,93	56,25	38,74	TE149	52,00	9,88	18,25	1,97	40,08	9,72	5,40	87,98	41,95
TE114	36,25	7,69	13,25	1,49	40,33	9,58	4,95	59,84	47,99	TE150	37,13	11,88	17,75	1,48	41,11	9,76	4,78	74,78	43,48
TE115	54,00	10,75	16,88	2,56	46,09	10,07	5,38	53,91	36,82	TE151	42,75	12,38	19,00	1,98	48,71	10,08	5,21	65,72	38,94
TE116	33,00	8,63	12,38	1,37	44,89	9,05	5,20	75,27	51,90	TE152	43,50	11,25	16,13	2,50	58,42	11,05	5,57	82,34	47,24
TE118	*	*	*	*	*	*	*	*	*	TE153	41,88	11,13	15,25	2,12	58,51	10,85	5,64	75,83	50,70
TE119	34,50	10,56	13,75	1,18	45,22	9,85	5,06	57,81	43,71	TE155	55,75	9,94	16,50	1,99	39,35	9,98	4,93	55,02	39,04
TE120	48,50	9,06	15,50	1,99	40,95	9,83	5,05	65,19	39,81	TE156	*	*	*	*	*	*	*	*	*
TE122	35,63	9,63	13,50	1,58	48,21	9,54	4,87	89,53	48,06	TE158	*	*	*	*	*	*	*	*	*

Appendix III Descriptive statistics of all traits evaluated on DH lines and the averages of the parental cultivars in four environments

Trait[a]	Environment	DH population (n = 139)			Parental lines	
		Mean ± SD	Min	Max	Tugela-DN	Elands
GWPS (g)	All	1.4 ± 0.4	0.4	2.4	1.8	1.4
	ARL18	0.9 ± 0.3	0.3	1.6	1.2	1.0
	BHM18	1.8 ± 0.6	0.1	3.2	2.0	1.9
	HAR18	1.3 ± 0.4	0.3	2.3	1.5	1.2
	BHM19	1.7 ± 0.4	0.8	2.3	2.3	1.5
SL (cm)	All	9.9 ± 1.2	7.1	13.7	9.9	10.1
	ARL18	8.2 ± 1.2	6.2	14.2	8.0	8.7
	BHM18	11.4 ± 1.2	8.4	14.3	11.8	11.8
	HAR18	10.5 ± 1.1	6.6	13.6	10.2	9.8
	BHM19	9.5 ± 1.2	7.0	12.6	9.6	9.9
SPS	All	14.8 ± 2.0	9.6	20.9	16.9	13.6
	ARL18	10.0 ± 1.8	6.5	17.9	11.1	10.2
	BHM18	18.0 ± 2.1	10.5	23.9	20.4	16.7
	HAR18	16.5 ± 1.8	12	21.8	18.4	14.1
	BHM19	14.8 ± 2.1	9.5	20	17.6	13.2
TKW (g)	All	33.6 ± 6.3	17.2	49.8	32.7	34.7
	ARL18	26.7 ± 5.4	14.6	41.3	27.6	31.3
	BHM18	37.0 ± 7.5	10.0	53.9	34.1	37.7
	HAR18	27.4 ± 7.7	11.0	45.4	23.6	29.1
	BHM19	43.2 ± 4.5	33.1	58.5	45.3	40.8
KNPS	All	41.2 ± 8.3	17.1	61.1	52.3	40.4
	ARL18	33.6 ± 7.8	16.2	57.6	39.7	33.2
	BHM18	48.5 ± 10.5	6.0	70.8	57.9	52.3
	HAR18	42.2 ± 7.7	24.5	58.6	57.2	38.3
	BHM19	40.6 ± 7.2	21.7	57.3	54.3	37.8
GL (mm)	All	9.6 ± 0.5	8.1	11.1	10.0	9.5
	ARL18	9.8 ± 0.5	8.4	11.1	10.0	9.7
	BHM18	9.0 ± 0.5	8.1	10.4	9.7	9.1
	HAR18	9.8 ± 0.6	7.5	11.6	10.3	9.7
	BHM19	9.7 ± 0.5	8.5	11.1	10.0	9.5
GW (mm)	All	4.8 ± 0.3	3.8	5.4	4.9	4.8
	ARL18	4.6 ± 0.3	3.5	5.5	4.7	4.6
	BHM18	4.7 ± 0.3	3.8	5.3	4.9	4.7
	HAR18	4.6 ± 0.4	3.5	5.3	4.8	4.9
	BHM19	5.1 ± 0.3	4.4	5.6	5.1	5.1
Fe (mg/kg)	All	63.2 ± 14.5	35.8	112.7	64.2	67.3
	ARL18	67.5 ± 13.3	43.1	111.1	67.3	67.3
	BHM18	47.8 ± 14.2	10.0	82.5	43.5	43.2
	HAR18	69.5 ± 15.9	44.6	120.1	60.2	69.6
	BHM19	68.0 ± 14.6	45.5	137.2	85.6	88.9
Zn (mg/kg)	All	50.7 ± 9.8	34.3	85.7	46.6	48.7
	ARL18	71.4 ± 14.8	45.9	130.9	56.3	63.6
	BHM18	40.2 ± 8.5	26.5	60.3	37.3	30.3
	HAR18	42.5 ± 8.5	28.1	70.4	44.4	50.4
	BHM19	48.5 ± 7.4	36.7	81.2	48.5	50.5

[a] Traits were defined in Table 4.1 (Chapter four)

Appendix IV Phenotypic correlation coefficient matrix among yield-related traits based on trait values in four environments in 2017 and 2018

Traits[a]	Env	KNPS	SL	SPS	GWPS	TKW	GL	GW	Fe
SL	ARL18	0.21	1						
	BHM18	**0.66**	1						
	HAR18	**0.44**	1						
	BHM19	0.36	1						
SPS	ARL18	**0.64**	**0.42**	1					
	BHM18	**0.81**	**0.75**	1					
	HAR18	**0.67**	**0.76**	1					
	BHM19	**0.58**	**0.74**	1					
GWPS	ARL18	**0.83**	0.16	**0.62**	1				
	BHM18	**0.69**	**0.48**	**0.54**	1				
	HAR18	**0.72**	**0.42**	**0.44**	1				
	BHM19	**0.78**	**0.39**	**0.46**	1				
TKW	ARL18	0.05	0.005	0.28	**0.50**	1			
	BHM18	0.24	0.17	0.17	**0.83**	1			
	HAR18	**0.41**	0.21	0.20	**0.85**	1			
	BHM19	-0.02	0.18	-0.04	**0.57**	1			
GL	ARL18	0.21	0.13	0.29	**0.48**	**0.56**	1		
	BHM18	0.16	0.11	0.12	**0.48**	**0.54**	1		
	HAR18	0.20	0.32	0.11	**0.60**	**0.46**	1		
	BHM19	-0.02	0.30	0.06	0.29	**0.49**	1		
GW	ARL18	0.13	-0.12	0.32	**0.38**	**0.67**	**0.63**	1	
	BHM18	0.17	-0.004	0.17	**0.48**	**0.57**	0.37	1	
	HAR18	0.12	0.20	0.04	**0.60**	**0.55**	**0.74**	1	
	BHM19	0.07	-0.001	-0.11	**0.51**	**0.71**	0.26	1	
Fe	ARL18	-0.11	-0.18	-0.20	-0.28	-0.33	-0.20	-0.24	1
	BHM18	0.29	0.22	0.18	0.25	0.11	-0.08	-0.17	1
	HAR18	0.20	-0.05	0.17	-0.07	-0.16	-0.02	-0.05	1
	BHM19	0.13	0.11	0.09	0.04	-0.10	0.12	-0.12	1
Zn	ARL18	-0.42	-0.28	-0.32	-0.50	-0.34	-0.16	-0.28	**0.51**
	BHM18	0.16	0.17	0.13	-0.06	-0.15	-0.06	-0.20	**0.52**
	HAR18	-0.24	-0.32	-0.25	-0.38	-0.28	-0.06	-0.13	0.34
	BHM19	-0.26	-0.04	-0.14	-0.04	-0.30	-0.07	-0.36	**0.47**

Values in red, green and black are strong, moderate and weak/no correlations among yield-related traits, respectively

[a] Traits were defined in Table 4.1 (Chapter four)

Appendix V Summary of QTLs identified for micronutrient and yield-related traits using 139 doubled haploids across environments in two seasons (2017/18 and 2018/19).

Traits[a]	QTL	Trial	Pos (cM)	Marker interval		LOD[b]	PVE (%)[c]	Add[d]
				Left	Right			
GWPS	QGwps.sg-1A.1a	BHM18	274.0	c1Am0043	c1Am0052	2.07	2.7	-0.22
	QGwps.sg-1A.1b	BHM18	278.0	c1Am0052		2.19	3.5	-0.13
	QGwps.sg-2B.1	BHM18	300.0	cUAm1319	cUAm1278	2.26	2.9	-0.27
	QGwps.sg-2D.1a	ARL18	584.0	cUAm1466		2.26	9.7	-0.07
	QGwps.sg-2D.1b	HAR18	316.0	cUAm1439	c2Dm0042	2.26	2.0	-0.09
	QGwps.sg-4A.1	**ARL18**	**60.7**	**c4Am0036**		**2.49**	**10.7**	**0.11**
	QGwps.sg-5B.1a*	ARL18	72.0	cUAm1428	c5Bm0069	2.19	15.4	0.11
	QGwps.sg-5B.1b	ARL18	74.0	cUAm1428	c5Bm0069	2.18	15.4	0.15
	QGwps.sg-5D.1a	ARL18	74.0	c5Dm0043	cUnkm0040	2.29	16.1	0.12
	QGwps.sg-5D.1b	ARL18	86.0	cUnkm0040	cUAm1314	2.15	15.2	0.12
	QGwps.sg-5D.1c	BHM18	90.0	cUAm1314	cUAm1178	1.94	17.8	-0.19
	QGwps.sg-5D.2d	BHM18/ BHM19	94.0/96.9	cUAm1314	cUAm1178	2.56/1.17	7.5/6.6	-0.24/-0.07
	QGwps.sg-6A.1a	BHM18	206.0	c6Am0013	c6Am0012	2.43	7.1	-0.32
	QGwps.sg-6A.1b	BHM18	212.0	c6Am0013	c6Am0012	2.37	7.5	-0.33
	QGwps.sg-6B.2	BHM18/BHM19	486.0	cUAm0759	c6Bm0023	2.52	5.0	-0.23

Pos – Position, cM – Centi Morgan, Env – Environment;

[a]Traits were defined in Table 4.1 (Chapter four);

[b]LOD=Logarithm of odds, where values are the peak logarithm of odds score for the given QTL;

[c]PVE=Phenotypic variance explained, where values indicate the phenotypic variation explained by the QTL;

[d]ADD=Additive, where values indicate the additive effect of the QTL; positive and negative effects indicate that the QTL alleles were contributed by Elands and Tugela-DN, respectively.

Traits[a]	QTL	Trial	Pos (cM)	Marker interval		LOD[b]	PVE (%)[c]	Add[d]
				Left	Right			
SL	QSl.sg-1D.1	HAR18	710.0	c1Dm0065	c1Dm0001	2.67	6.2	0.55
	QSl.sg-2D.1	BHM19	519.3	c2Dm0106		2.12	1.4	-0.15
	QSl.sg-3D.1	BHM18	336.5	c3Dm0024	cUAm1613	2.78	13.8	-0.72
	QSl.sg-4A.1	BHM19	402.7	c5Dm0033	c4Am0029	2.21	5.1	-0.23
	QSl.sg-4B.1	BHM19	118.0	c4Bm0018	c4Bm0009	2.23	2.5	-0.80
	QSl.sg-5D.1	BHM18	221.4	c5Dm0033		2.64	13.1	0.40
	QSl.sg-6A.1	ARL18	294.0	c6Am0012	c6Am0032	2.35	16.5	1.11
	QSl.sg-7A.1	HAR18	190.0	c7Am0065	cUAm0764	2.55	11.4	0.88
	QSl.sg-7B.2	BHM18/ BHM19	574.2	c7Bm0073		1.59	11.1	0.31
SPS	QSps.sg-3A.1	HAR18		c3Am0014	c3A0070	2.41	7.2	-0.52
	QSps.sg-4A.1*	**ARL18**	**60.7**	**c4Am0036**		**2.48**	**13.4**	**0.64**
	QSps.sg-5B.2a*	ARL18/BHM18	72.0	cUAm1428	c5Bm0069	2.24	4.8	0.93
	QSps.sg-5B.1b*	ARL18	74.0	cUAm1428	c5Bm0069	2.25	1.82	0.88
	QSps.sg-5D.1a	ARL18	44.0	cUAm0974	c5Dm0043	2.29	16.1	1.06
	QSps.sg-5D.1b	ARL18	74.0	c5Dm0043	cUnkm0040	1.89	15.8	0.66
	QSps.sg-5D.1c	BHM18	90.0	cUAm1314	cUAm1178	2.47	3.5	-0.47
	QSps.sg-5D.1d	BHM18	94.0	cUAm1314	cUAm1178	2.02	1.1	-1.05
	QSps.sg-6B.1a	ARL18	12.0	c6Bm0015	c6Bm0051	1.80	6.6	0.83
	QSps.sg-6B.1b	BHM18	246.0	cUAm1018	c6Bm0019	2.20	12.4	-1.77
	QSps.sg-7A.1	HAR18	192.0	c7Am0065	cUAm0764	2.59	8.7	1.198
	QSps.sg-7B.1	BHM19	148.0	cUAm0995	c7Bm0063	1.63	11.8	0.92

Pos – Position, cM – Centi Morgan, Env – Environment;

[a]Traits were defined in Table 4.1 (Chapter four);

[b]LOD=Logarithm of odds, where values are the peak logarithm of odds score for the given QTL;

[c]PVE=Phenotypic variance explained, where values indicate the phenotypic variation explained by the QTL;

[d]ADD=Additive, where values indicate the additive effect of the QTL; positive and negative effects indicate that the QTL alleles were contributed by Elands and Tugela-DN, respectively.

Traits[a]	QTL	Trial	Pos (cM)	Marker interval		LOD[b]	PVE (%)[c]	Add[d]
				Left	Right			
KNPS	QKnps.sg-1A.1	HAR18	64.0	c1Am0019	c1Am0012	2.11	7.2	-3.30
	QKnps.sg-2B.1	BHM18	3.8	cUAm0805		2.08	6.8	2.08
	QKnps.sg-4A.1	**ARL18**	**60.7**	**c4Am0036**		**3.81**	**19.5**	**3.36**
	QKnps.sg-5B.1a*	BHM18	68.0	cUAm1428	c5Bm0069	1.76	2.8	-1.46
	QKnps.sg-5B.1b	ARL18	98.0	c5Bm0069	c5Bm0087	1.89	1.3	3.95
	QKnps.sg-5D.1a	ARL18	74.0	c5Dm0043	cUnkm0040	2.22	15.7	3.56
	QKnps.sg-5D.1b	ARL18	86.0	cUnkm0040	cUAm1314	2.33	16.4	3.47
	QKnps.sg-5D.1c	BHM18	90.0	cUAm1314	cUAm1178	3.21	17.7	-0.18
	QKnps.sg-5D.2d	BHM18/BHM19	94.0/96.0	cUAm1314	cUAm1178	3.73/1.83	20.2/8.4	-4.29/-1.91
	QKnps.sg-6B.1	ARL18	6.0	c6Bm0015	c6Bm0051	1.86	13.3	3.66
TKW	QTkw.sg-1A.1a	BHM18	274.0	c1Am0043	c1Am0052	2.05	11.7	-3.10
	QTkw.sg-1A.1b	BHM18	278.0	c1Am0052		2.03	10.1	-2.89
	QTkw.sg-2A.1	HAR18	530.0	cUAm0433		1.69	7.7	2.35
	QTkw.sg-2D.1	ARL18	334.0	cUAm1439	c2Dm0042	2.06	14.6	-2.56
	QTkw.sg-4B.1	ARL18	186.0	c4Bm0009		2.28	13.1	2.19
	QTkw.sg-5B.1	BHM18	266.0	c5Bm0024	c5Bm0074	2.31	10.4	-2.79
	QTkw.sg-6A.1a	BHM18	206.0	c6Am0013	c6Am0012	2.45	13.8	-4.52
	QTkw.sg-6A.1b	BHM18	212.0	c6Am0013	c6Am0012	2.53	14.2	-1.56
	QTkw.sg-6D.1a	BHM18	455.3	c6Dm0021		2.04	6.2	-3.09
	QTkw.sg-6D.1b	BHM19	188.0	c6Dm0011	cUAm1161	2.09	5.2	1.27

Pos – Position, cM – Centi Morgan, Env – Environment;

[a]Traits were defined in Table 4.1 (Chapter four);

[b]LOD=Logarithm of odds, where values are the peak logarithm of odds score for the given QTL;

[c]PVE=Phenotypic variance explained, where values indicate the phenotypic variation explained by the QTL;

[d]ADD=Additive, where values indicate the additive effect of the QTL; positive and negative effects indicate that the QTL alleles were contributed by Elands and Tugela-DN, respectively.

Traits[a]	QTL	Trial	Pos (cM)	Marker interval		LOD[b]	PVE (%)[c]	Add[d]
				Left	Right			
GL	QGl.sg-2A.1	ARL18	486.0	c2Am0076	c2Am0009	1.95	2.1	-0.18
	QGl.sg-2D.1	ARL18	561.5	cUAm1555		1.98	2.3	0.09
	QGl.sg-3A.3	BHM19/BHM18/ARL18	42.0/60.0/84.0	c3Am0014	c3Am0070	2.62/1.75/1.69	9.1/8.0/8.9	0.40/0.37/0.25
	QGl.sg-5B.1	ARL18	250.0	c5Bm0074	c5Bm0081	1.86	13.9	-0.30
	QGl.sg-5D.1	HAR18	572.0	c5Dm0048	c5Dm0007	2.23	20.7	-0.33
GW	QGw.sg-2D.1a	BHM18	334.0	cUAm1439	c2Dm0042	1.84	13.0	-0.14
	QGw.sg-2D.1b	BHM19	392.0	c2Dm0074	c2Dm0110	2.73	3.6	-0.07
	QGw.sg-3A.1	ARL18	97.1	c3Am0070		2.27	14.4	0.15
	QGw.sg-5B.1a	ARL18	58.0	cUAm1428	c5Bm0069	2.02	11.9	0.22
	QGw.sg-5B.1b	HAR18	92.0	c5Bm0069	c5Bm0087	2.02	4.8	-0.18
	QGw.sg-5D.1	HAR18	394.0	c5Dm0004	c5Dm0050	1.96	7.7	-0.17
	QGw.sg-6A.1	BHM18	92.0	cUAm0425	c6Am0024	2.02	5.4	-0.34
	QGw.sg-6D.1a	BHM18/HAR18	476.0	c6Dm0021	c6Dm0006	1.89	5.7	-0.09
Fe	QFe.sg-2D.1a	ARL18	295.4	cUAm1439		2.91	20.9	-6.24
	QFe.sg-2D.1b	ARL18	298.0	cUAm1439	c2Dm0042	2.91	21.0	-6.61
	QFe.sg-5B.1	ARL18	108.0	c5Bm0069	c5Bm0087	1.84	4.4	-7.51
	QFe.sg-5D.1	ARL18	176.0	c5Dm0016	c5Dm0003	1.92	1.5	-5.22
	QFe.sg-7D.1	BHM18/HAR18	44.0	c7Dm0047	c7Dm0041	2.21	20.2	-13.01

Zn	QZn.sg-2D.1a	ARL18	295.4	cUAm1439		2.65	19.3	-5.88
	QZn.sg-2D.1b	ARL18	298.0	cUAm1439	c2Dm0042	2.57	18.8	-6.13
	QZn.sg-5B.1	BHM19	50.0	cUAm1428	c5Bm0069	2.31	14.5	-5.27
	QZn.sg-5D.1	ARL18	176.0	c5Dm0016	c5Dm0003	2.02	15.1	-5.23
	QZn.sg-6A.1	BHM18/HAR18	88.4	cUAm0425	c6Am0024	1.71	16.0	7.39
	QZn.sg-6B.1	ARL18	0.0	c6Bm0015		2.82	7.2	-6.19

Pos – Position, cM – Centi Morgan, Env – Environment;

[a]Traits were defined in Table 4.1 (Chapter four);

[b]LOD=Logarithm of odds, where values are the peak logarithm of odds score for the given QTL;

[c]PVE=Phenotypic variance explained, where values indicate the phenotypic variation explained by the QTL;

[d]ADD=Additive, where values indicate the additive effect of the QTL; positive and negative effects indicate that the QTL alleles were contributed by Elands and Tugela-DN, respectively.

Appendix VI List of publications and conferences attended

Publication:

Book chapter: Lephuthing, M.C., Baloyi, T.A., Sosibo, N.Z. and Tsilo, T.J., 2017. Chapter 5: Progress and Challenges in Improving Nutritional Quality in Wheat. In Wangera, R. and Owuoche, J. (Eds). *Wheat Improvement, Management and Utilization*, InTech, Croatia. Publisher: InTech, ISBN 978-953-51-3151-9. 77-95.

Conference attended and presentations:

1. Cereal Science and Technology (September 11, 2019, Grain Building, Pretoria)

Oral: Genetic analysis of yield component traits in South African bread wheat (*Triticum aestivum* L.) genotypes

Authors: M.C. Lephuthing, V.L. Tolmay, T.A. Baloyi and T.J. Tsilo

2. African Combined Congress (January 14-18, 2018, Ratanga Junction, Cape Town)

Poster: Improving nutritional quality of wheat: What is important in South African context?

Authors: M.C. Lephuthing, V.L. Tolmay, T.A. Baloyi, N.Z., Sosibo, and T.J. Tsilo